



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: <b>PLANT CELLULOSE SYNTHASES</b>		<p><b>Published</b>  <i>Without international search report and to be republished upon receipt of that report.</i></p>	
(57) Abstract		<p>This invention relates to an isolated nucleic acid fragment encoding a cellulose synthase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the cellulose synthase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the cellulose synthase in a transformed host cell.</p>	
<pre> SEQ ID NO:2      1 SEQ ID NO:4      RAAGAGCRRGGKKQQPEQQKLASVSLP-LPSSRFTIPTPPRKTQH--RFLACFG--I SEQ ID NO:6      RSTTTKERSLAAQPRAAPQNPQPF--ATACACERSFRPGDQRNGLRAFRCAAAGTV SEQ ID NO:8      RCB--RWTTCSSPPPTPTGRRSPRTP- SEQ ID NO:10     SEQ ID NO:12 SEQ ID NO:14     SEQ ID NO:16 SEQ ID NO:16     SEQ ID NO:18 SEQ ID NO:18     SEQ ID NO:20 SEQ ID NO:20     SEQ ID NO:22 SEQ ID NO:22     SEQ ID NO:23 SEQ ID NO:23     SEQ ID NO:24 SEQ ID NO:24     MFTYGR- SEQ ID NO:25     SEQ ID NO:26 SEQ ID NO:26     RASTPPOTSKXVRNSUSGSQYVXPARYTTSBRGVVLS-RKHELSKGELGQDVNTYVLP SEQ ID NO:27     SEQ ID NO:28 SEQ ID NO:28     SEQ ID NO:29 SEQ ID NO:29     R---PR- </pre> <pre> 60 SEQ ID NO:1      - SEQ ID NO:2      -M-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:4      -R-REGARG---M-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:6      -RERUPAGRGGGFENIE-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:8      -RERUPAGRGGGFENIE-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:10     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:12     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:14     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:16     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:18     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:20     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:22     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:23     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:24     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:25     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:26     PFPOMOPHATKAEGGTQVNLKELFTGGPFRVTRMLNDKVIDAVVTFPOMCAGKHZSCAMP SEQ ID NO:27     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:28     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- SEQ ID NO:29     -R-ASAGLVAGSGEMMELV-VIERRDGEPGPKP--MDQRMGQVQCI- </pre> <pre> 61 SEQ ID NO:1      - 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SEQ ID NO:2      CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:4      CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:6      CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:8      CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:10     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:12     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:14     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:16     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:18     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:20     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:22     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:23     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:24     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:25     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- SEQ ID NO:26     CGDGVGLMPDPGEPPVACHECAFPICRDCCTEYERKEDQUTQNCPCQCYTPERLADCGAVVGD- </pre>			

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TITLE

## PLANT CELLULOSE SYNTHASES

This application claims the benefit of U.S. Provisional Application No. 60/092,844.  
filed July 14, 1998.

5

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding cellulose biosynthetic enzymes in plants and seeds.

BACKGROUND OF THE INVENTION

10 Cellulose is a major component of plant fiber, e.g. cotton fiber. Cellulose is composed of crystalline beta-1,4-glucan microfibrils (see World Patent Publication No. WO 98/00549). These microfibrils are strong and can resist enzymatic and mechanical degradation and are important in determining nutritional quality of animal and human foodstuffs. Hence, modification of the biosynthetic pathway responsible for cellulose  
15 synthesis through modification of cellulose synthase activity could potentially alter fiber quantity, either by producing more or less fiber in a particular plant species or in a specific organ or tissue of a particular plant. Modification of cellulose synthase activity could increase the value of the fiber to the end-user and may improve the structural integrity of the plant cell wall. Lastly, because cellulose is a major cell wall component, inhibition of  
20 cellulose synthesis would probably be lethal. Thus, cellulose synthase may serve as the target for a novel class of herbicides. Plant cellulose synthase genes, homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase, have been reported from cotton, *Arabidopsis*, rice and alfalfa (World Patent Publication Nos. WO 98/00549 and WO 98/18949).

25 There is a great deal of interest in identifying the genes that encode proteins involved in cellulose synthesis. These genes may be used in plant cells to control the synthesis of cellulose. Accordingly, the availability of nucleic acid sequences encoding all or a portion of a cellulose synthase would facilitate studies to better understand cellulose synthesis in plants and provide genetic tools to alter cellulose production.

30

SUMMARY OF THE INVENTION

The instant invention relates to isolated nucleic acid fragments encoding cellulose biosynthesis enzymes. Specifically, this invention concerns an isolated nucleic acid fragment encoding a cellulose synthase and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding a cellulose synthase. In  
35 addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding cellulose synthase. An additional embodiment of the instant invention pertains to a polypeptide encoding all or a substantial portion of a cellulose synthase.

In another embodiment, the instant invention relates to a chimeric gene encoding a cellulose synthase, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production 5 of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a cellulose synthase, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of 10 altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the 15 level of expression of a cellulose synthase in a transformed host cell comprising:  
a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of cellulose synthase in the transformed host cell.

An addition embodiment of the instant invention concerns a method for obtaining a 20 nucleic acid fragment encoding all or a substantial portion of an amino acid sequence encoding a cellulose synthase.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a cellulose synthase, the method comprising 25 the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of cellulose synthase in the transformed host cell; (c) optionally purifying the cellulose synthase 30 expressed by the transformed host cell; (d) treating the cellulose synthase with a compound to be tested; and (e) comparing the activity of the cellulose synthase that has been treated with a test compound to the activity of an untreated cellulose synthase, thereby selecting compounds with potential for inhibitory activity.

**35 BRIEF DESCRIPTION OF THE  
DRAWINGS AND SEQUENCE DESCRIPTIONS**

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figure 1 shows a comparison of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* sequences (SEQ ID NOs:23 (gi 2827139), 24 (gi 2827141), 26 (gi 4467125), 27 (gi 4886756) and 29 (gi 3135611)) and *Gossypium hirsutum* sequences (SEQ ID NOs:25 (gi 1706958) and 28 (gi 5081779)).

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1  
Cellulose Biosynthetic Enzymes

Protein	Clone Designation	SEQ ID NO: (Nucleotide)      (Amino Acid)	
Cellulose Synthase	bsh1.pk0002.f6	1	2
Cellulose Synthase	Contig composed of: cc01n.pk0005.g3 cdt2c.pk002.g1 cdt2c.pk002.l16 csc1c.pk002.i1 p0031.ccmar05rb p0110.cgsma57r	3	4
Cellulose Synthase	cr1n.pk0135.e10	5	6
Cellulose Synthase	p0097.cqrard17rc	7	8
Cellulose Synthase	p0122.ckamh70rc	9	10
Cellulose Synthase	r1r24.pk0073.g1	11	12
Cellulose Synthase	sdp2c.pk005.o22	13	14
Cellulose Synthase	ses8w.pk0028.f3	15	16
Cellulose Synthase	ssl.pk0036.c10	17	18
Cellulose Synthase	Contig composed of: wl1.pk0009.c9 wr1.pk0160.d11 wre1n.pk0043.f9 wre1n.pk0043.h8 wre1n.pk0131.g10	19	20
Cellulose Synthase	wl1n.pk0044.b1	21	22

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The

symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, a  
5 "nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded,  
optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid  
fragment in the form of a polymer of DNA may be comprised of one or more segments of  
cDNA, genomic DNA or synthetic DNA.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or  
10 more constituent nucleotide sequences that share common or overlapping regions of  
sequence homology. For example, the nucleotide sequences of two or more nucleic acid  
fragments can be compared and aligned in order to identify common or overlapping  
sequences. Where common or overlapping sequences exist between two or more nucleic  
acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be  
15 assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein  
changes in one or more nucleotide bases results in substitution of one or more amino acids,  
but do not affect the functional properties of the polypeptide encoded by the nucleotide  
sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in  
20 one or more nucleotide bases does not affect the ability of the nucleic acid fragment to  
mediate alteration of gene expression by gene silencing through for example antisense or co-  
suppression technology. "Substantially similar" also refers to modifications of the nucleic  
acid fragments of the instant invention such as deletion or insertion of one or more  
nucleotides that do not substantially affect the functional properties of the resulting  
25 transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional  
properties of the resulting protein molecule. It is therefore understood that the invention  
encompasses more than the specific exemplary nucleotide or amino acid sequences and  
includes functional equivalents thereof.

For example, it is well known in the art that antisense suppression and co-suppression  
30 of gene expression may be accomplished using nucleic acid fragments representing less than  
the entire coding region of a gene, and by nucleic acid fragments that do not share 100%  
sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid  
fragment which result in the production of a chemically equivalent amino acid at a given  
site, but do not effect the functional properties of the encoded polypeptide, are well known in  
35 the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be  
substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more  
hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result  
in substitution of one negatively charged residue for another, such as aspartic acid for

glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed 5 modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the nucleic acid fragments disclosed herein.

10 Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Preferred are those nucleic acid fragments whose nucleotide sequences encode amino acid sequences that are 80% identical to the amino acid sequences 15 reported herein. More preferred nucleic acid fragments encode amino acid sequences that are 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite 20 (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

25 A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST 30 (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes 35 comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular

nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that

5 comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

10 "Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited  
15 by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

20 "Synthetic nucleic acid fragments" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. "Chemically synthesized", as related to nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments  
25 may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards  
30 those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

35 "Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived

from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

5 "Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, 10 and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a

15 promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed 20 of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions.

Promoters which cause a nucleic acid fragment to be expressed in most cell types at most 25 times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have 30 identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, 35 mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences

encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

5 "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is

10 without introns and that can be translated into polypeptide by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. "Antisense RNA" refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the

15 expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

20 The term "operably linked" refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to

25 regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of

30 suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

35 "Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed.

"Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53).

5 If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

10 15 "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation

20 25 technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of a cellulose synthase enzyme have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other cellulose synthase enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing

methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling,  
5 nick translation, or end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate  
10 stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer  
15 is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998) to generate  
20 cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673; Loh et al. (1989) *Science* 243:217). Products generated by the 3'  
25 and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be  
30 used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of cellulose synthase in those cells.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

5 Plasmid vectors comprising the instant chimeric gene can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. 10 (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired 15 expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different 20 cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant 25 Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the 30 instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in 35 reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U. S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds, and is not an inherent part of the invention. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded cellulose synthase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

Additionally, the instant polypeptides can be used as targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptides described herein catalyze a step in the synthesis of cellulose. Accordingly, inhibition of the activity of one or more of the enzymes described 5 herein could lead to inhibition plant growth. Thus, the instant polypeptides could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant 10 breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. 15 (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map 20 previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the 25 methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used 30 for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask 35 (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al. (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides.

Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

#### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without

departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

5 cDNA libraries representing mRNAs from various barley, corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2  
cDNA Libraries from Barley, Corn, Rice, Soybean and Wheat

Library	Tissue	Clone
bsh1	Barley ( <i>Hordeum vulgare</i> ) sheath, developing seedling	bsh1.pk0002.f6
ccoln	Corn ( <i>Zea mays</i> ) cob of 67 day old plants grown in green house*	ccoln.pk0005.g3
cdt2c	Corn ( <i>Zea mays</i> ) developing tassel 2	cdt2c.pk002.g1 cdt2c.pk002.l16
cr1n	Corn ( <i>Zea mays</i> ) root from 7 day seedlings grown in light*	cr1n.pk0135.e10
csc1c	Corn ( <i>Zea mays</i> ) 20 day seedling (germination under cold stress)	csc1c.pk002.i1
p0031	Corn ( <i>Zea mays</i> ) shoot culture, initiated from seed derived meristems culture was maintained on 273N medium.	p0031.ccmar05rb
p0110	Corn ( <i>Zea mays</i> ) stages V3/V4** leaf tissue minus midrib harvested 4 hours, 24 hours and 7 days after infiltration with salicylic acid, tissues pooled*	p0110.cgsma57r
p0097	Corn ( <i>Zea mays</i> ) stage V9** whorl section (7 cm) from plant infected four times with european corn borer	p0097.cqrad17rc
p0122	Corn ( <i>Zea mays</i> ) pith tissue collected from internode subtending ear node 5 days after pollination	p0122.ckamh70rc
rlr24	Rice ( <i>Oryza sativa</i> ) leaf (15 days after germination) 24 hours after infection of <i>Magaporthe grisea</i> strain 4360-R-62 (AVR2-YAMO); Resistant	rlr24.pk0073.g1
sdp2c	Soybean ( <i>Glycine max</i> ) developing pods 6-7 mm	sdp2c.pk005.o22
ses8w	Soybean ( <i>Glycine max</i> ) mature embryo 8 weeks after subculture	ses8w.pk0028.f3
ss1	Soybean ( <i>Glycine max</i> ) seedling 5-10 day	ssl.pk0036.c10
wl1	Wheat ( <i>Triticum aestivum</i> ) leaf 7 day old seedling, light grown	wl1.pk0009.c9
wl1n	Wheat ( <i>Triticum aestivum</i> ) leaf 7 day old seedling, light grown*	wl1n.pk0044.b1
wr1	Wheat ( <i>Triticum aestivum</i> ) root; 7 day old seedling, light grown	wr1.pk0160.d11

Library	Tissue	Clone
wre1n	Wheat ( <i>Triticum aestivum</i> ) root; 7 day old etiolated seedling*	wre1n.pk0043.f9
		wre1n.pk0043.h8
		wre1n.pk0131.g10

\*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

\*\*V3, V4 and V9 refer to stages of corn growth. The descriptions can be found in "How a Corn Plant Develops" Special Report No. 48, Iowa State University of Science and Technology Cooperative Extension Service Ames, Iowa, Reprinted February 1993.

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA 10 libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA 15 ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted 20 cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### EXAMPLE 2

25 Identification of cDNA Clones

cDNA clones encoding cellulose synthase enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS 30 translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The

DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the “nr” database using the BLASTX algorithm (Gish and States (1993) *Nature Genetics* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a

5 sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as “pLog” values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST “hit” represent homologous proteins.

EXAMPLE 3

10 Characterization of cDNA Clones Encoding Cellulose Synthase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to cellulose synthase from *Arabidopsis thaliana* (NCBI Identifier No. gi 2827139, gi 2827141, gi 4467125, gi 4886756 and gi 3135611) and *Gossypium hirsutum* (NCBI Identifier No. gi 1706958 and 5081779). Shown 15 in Table 3 are the BLAST results for individual ESTs (“EST”), the sequences of the entire cDNA inserts comprising the indicated cDNA clones (“FIS”), complete gene sequences (“CGS”) or contigs assembled from two or more ESTs (“Contig”):

TABLE 3

20 BLAST Results for Sequences Encoding Polypeptides Homologous to *Arabidopsis thaliana* and *Gossypium hirsutum* Cellulose Synthase

Clone	Status	BLAST pLog Score
bsh1.pk0002.f6	FIS	154.00 (gi 2827139)
Contig composed of:	Contig	>254.00 (gi 2827141)
cc1n.pk0005.g3		
cdt2c.pk002.g1		
cdt2c.pk002.l16		
csc1c.pk002.i1		
p0031.ccmar05rb		
p0110.cgsma57r		
cr1n.pk0135.e10	FIS	176.00 (gi 1706958)
p0097.cqrard17rc	CGS	>254.00 (gi 2827141)
p0122.ckamh70rc	CGS	>254.00 (gi 2827141)
r1r24.pk0073.g1	EST	77.70 (gi 4467125)
sdp2c.pk005.o22	FIS	>254.00 (gi 4886756)
ses8w.pk0028.f3	EST	>254.00 (gi 2827139)
ssl.pk0036.c10	EST	>254.00 (gi 2827141)
Contig composed of:	Contig	>254.00 (gi 5081779)
w11.pk0009.c9		
wr1.pk0160.d11		
wre1n.pk0043.f9		

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wre1n.pk0043.h8 wre1n.pk0131.g10		
wl1n.pk0044.b1	EST	166.00 (gi 3135611)

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Figure 1 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* (SEQ ID NOs:23 (gi 2827139), 24 (gi 2827141), 26 (gi 4467125), 27 (gi 4886756) and 29 (gi 3135611)) and 5 *Gossypium hirsutum* (SEQ ID NOs:25 (gi 1706958) and 28 (gi 5081779)) sequences. The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 and the *Arabidopsis thaliana* (SEQ ID NOs:23, 24, 26, 27 and 29) and *Gossypium hirsutum* (SEQ ID NOs:25 and 28) sequences.

10

**TABLE 4**

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Arabidopsis thaliana* and *Gossypium hirsutum* Cellulose Synthase

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SEQ ID NO.	Percent Identity to
2	82% (gi 2827139)
4	69% (gi 2827141)
6	89% (gi 1706958)
8	70% (gi 2827141)
10	70% (gi 2827141)
12	36% (gi 4467125)
14	86% (gi 4886756)
16	88% (gi 2827139)
18	86% (gi 2827141)
20	87% (gi 5081779)
22	70% (gi 3135611)

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15

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default 20 parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones

encode a substantial portion of a cellulose synthase. These sequences represent the first barley, corn, rice, soybean and wheat sequences encoding cellulose synthase.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

5 A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites  
10 (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the  
15 plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector  
20 pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit;  
25 U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses  
30 derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic  
35 proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers  
5 resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used  
10 to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After  
15 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 µL of ethanol. An aliquot (5 µL) of the DNA-coated  
20 gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of  
25 about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

30 Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the  
35 selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the

tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

5 A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about  
10 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

20 Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and  
25 placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with fluorescent lights on a 16:8 hour day/night schedule.  
30 Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Patent No. 4,945,050). A DuPont Biostatic™ PDS1000/HE instrument (helium retrofit) can be used  
35 for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225

(from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then  
5 be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70%  
10 ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette.

15 For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

20 Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into  
25 individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 6

##### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in  
35 pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using

oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve

5 GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase

10 (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent

15 cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

#### EXAMPLE 7

##### Evaluating Compounds for Their Ability to Inhibit the Activity of Cellulose Synthase

35 The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant

polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags.

Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide

5 ("(His)<sub>6</sub>"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

10 Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography,

15 wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the

20 C-terminus of thioredoxin. In addition, a (His)<sub>6</sub> peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be

25 accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β-mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™

30 affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for cellulose synthase activity are presented in WO 98/18949 and WO 98/00549.

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment comprising at least 900 nucleotides, wherein the nucleic acid fragment encodes a cellulose synthase comprising a member selected from the group consisting of:
  - (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 6, 12, 14, 16, 18, 20 and 22;
  - (b) an isolated nucleic acid fragment that is complementary to (a).
2. The isolated nucleic acid fragment of Claim 1 wherein nucleic acid fragment is a functional RNA.
3. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:1, 5, 11, 13, 15, 17, 19 and 21.
4. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.
5. A transformed host cell comprising the chimeric gene of Claim 4.
6. A cellulose synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2, 6, 12, 14, 16, 18, 20 and 22.
7. An isolated nucleic acid fragment encoding a cellulose synthase comprising a member selected from the group consisting of:
  - (a) an isolated nucleic acid fragment encoding an amino acid sequence that is functionally active polypeptide and at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:4, 8 and 10 ;
  - (b) an isolated nucleic acid fragment that is complementary to (a).
8. The isolated nucleic acid fragment of Claim 7 wherein nucleic acid fragment is a functional RNA.
9. The isolated nucleic acid fragment of Claim 7 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO:3, 7 and 9.
10. A chimeric gene comprising the nucleic acid fragment of Claim 7 operably linked to suitable regulatory sequences.
11. A transformed host cell comprising the chimeric gene of Claim 10.

12. A cellulose synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:4, 8, 10.

13. A method of altering the level of expression of a cellulose synthase in a host  
5 cell comprising:

- (a) transforming a host cell with the chimeric gene of any of Claims 4 and 10; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

10 wherein expression of the chimeric gene results in production of altered levels of a cellulose synthase in the transformed host cell.

14. A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a cellulose synthase comprising:

- (a) probing a cDNA or genomic library with the nucleic acid fragment of any of Claims 1 and 7;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment any of of Claims 1 and 7;
- (c) isolating the DNA clone identified in step (b); and
- (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding a cellulose synthase.

15. A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding a cellulose synthase comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21; and
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences 30 of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding a cellulose synthase.

16. The product of the method of Claim 14.

17. The product of the method of Claim 15.

35 18. A method for evaluating at least one compound for its ability to inhibit the activity of a cellulose synthase, the method comprising the steps of:

- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a cellulose synthase, operably linked to suitable regulatory sequences;
- 5 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the cellulose synthase encoded by the operably linked nucleic acid fragment in the transformed host cell;
- (c) optionally purifying the cellulose synthase expressed by the transformed host cell;
- 10 (d) treating the cellulose synthase with a compound to be tested; and
- (e) comparing the activity of the cellulose synthase that has been treated with a test compound to the activity of an untreated cellulose synthase, thereby selecting compounds with potential for inhibitory activity.

**Figure 1**

Figure 1 (cont'd.)

SEQ ID NO:27	CGDQIGLTVEGDLFVACNECGFPACRPCYEYERREGTQNCPQCKTRYKRLRGSPRVEGDE	
SEQ ID NO:28	-----	
SEQ ID NO:29	CRDEIELTVDGEPEFVACNECAFVCRPCYEYERREGNQACPQCKTRFKRLKGSPRVEGD-	
 181		
SEQ ID NO:2	-----	240
SEQ ID NO:4	EEEDGVDDLENEFNWSDK-----HDSQYLAESMLAHMSYG-RGADLDGVPQPFHPIPNVP	
SEQ ID NO:6	-----	
SEQ ID NO:8	EEEDGVDDLEGEFGLQDGAAHEDDPQYVAEMLRAQMSYG-RGGDAH---PGFSPVPNVP	
SEQ ID NO:10	EEEDGVDDLDNEFNW-DG-----HDSQSVAEMLYGHMSYG-RGGDPNGAPQAFQLNPNVP	
SEQ ID NO:12	-----	
SEQ ID NO:14	DEEDV-DDIEHEFNIDEQKNKHGQ---VAEAMLHGRMSYG--RGPEDDDNSQFPTPVIAG	
SEQ ID NO:16	-----	
SEQ ID NO:18	-----	
SEQ ID NO:20	-----	
SEQ ID NO:22	-----	P
SEQ ID NO:23	DEDDV-DDIENEFNYAQGANKARH---QRHGE---EFSSS---SRHESQPIPLLTHGHTVS	
SEQ ID NO:24	EEEEDIDDLLEYEFD-----HGMDEHAAEAALSSRLNTG--RGGLDSAPPG---SQIP	
SEQ ID NO:25	-----	
SEQ ID NO:26	-----	
SEQ ID NO:27	DEEDI-DDIEYEFNIEHEQDKHKH---SAEAMLYGKMSYG--RGPEDDENGRFP-PVIAG	
SEQ ID NO:28	-----	
SEQ ID NO:29	EEDDDIDDLNEFEYGN---NGIGFDQVSEGMSISRNSGFPQSDLDSAPPG---SQIP	
 241		
SEQ ID NO:2	-----	300
SEQ ID NO:4	LLTNGQMVDIIPPQHALVPSFV---GGGGKRIHPLPYADPNLPVQPRSMDPSKDLAAYG	
SEQ ID NO:6	-----	
SEQ ID NO:8	LLTNGQMVDIIPPEQHALVPSYMSGGGGGGKRIHPLPFADPNLPVQPRSMDPSKDLAAYG	
SEQ ID NO:10	LLTNGQMVDIIPPEQHALVPSFM---GGGGKRIHPLPYADPSLPVQPRSMDPSKDLAAYG	
SEQ ID NO:12	-----	
SEQ ID NO:14	GRSR---PVSGEFPISSNAYGDQMLSSSLHKRVHPYPVSEPGSARW---DEKKXDG	
SEQ ID NO:16	-----	
SEQ ID NO:18	-----	
SEQ ID NO:20	-----	
SEQ ID NO:22	LLTNGQMVDIIPPEQHALVPSYMSGGGGGGKRIHPLPFADPNLPVQPRSMDPSKDLAAYG	
SEQ ID NO:23	GEIRTPDTQSVRTTSGPLGPSDRNAISSPYIDPR-QPVPVRIVDPSK---DLNSYG	
SEQ ID NO:24	LLTYCDEDADMYSDRHALIVP--PS-TGYGNRVYPAPFTDSSAPPQARSMVPQKDIAEYG	
SEQ ID NO:25	-----	
SEQ ID NO:26	-----	ETQGTYG
SEQ ID NO:27	GHS---GEFPVGG-GYGNNG---EHGLHKRVHPYPSEAGS---EGG	
SEQ ID NO:28	-----	
SEQ ID NO:29	LLTYGDEDVEISSDRHALIVP--PSLGGHGNRVPVSLDPTVAHRRLMVPQKDLAVYG	
 301		
SEQ ID NO:2	-----	360
SEQ ID NO:4	YGSVAWKERMESWKQKQ-ERMHQTRNDGGGD-----DGDDADLPLM-DEARQPLSR	
SEQ ID NO:6	-----	
SEQ ID NO:8	YGSVAWKERMEGWKQKQ-ERLQHVRSEGGGDW-----DGDDADLPLM-DEARQPLSR	
SEQ ID NO:10	YGSVAWKERMENWKQRQ-ERMHQTRNDGGGD-----DGDDADLPLM-DEARQPLSR	
SEQ ID NO:12	-----	
SEQ ID NO:14	-----	
SEQ ID NO:16	-----	
SEQ ID NO:18	-----	HE
SEQ ID NO:20	-----	
SEQ ID NO:22	YGSVAWKERMEGWKQKQ-ERLQHVRSEGGGDW-----DGDDADLPLM-DEARQPLSR	
SEQ ID NO:23	LGNVDWKERVEGWKLQEKNMQLQMTGKYHEGKGG-EIEGTGSNGEELQM-ADDTRLPMR	

Figure 1 (cont'd.)

SEQ ID NO:24	YGSVAWKDRMEVWKRQGEKLQVIKHEGGNNRGSN-DDDELDDPDMPMM-DEGRQPLSR	
SEQ ID NO:25	-----	
SEQ ID NO:26	YGNAYWP-----QDEMYGD-----DMDEGMRRGMVETADKPWRPLSR	
SEQ ID NO:27	-----WRERMDDWKLQHG-----NLGPEPDDDPEMGLI-DEARQPLSR	
SEQ ID NO:28	-----	
SEQ ID NO:29	YGSVAWKDRMEEWKRKQNEKLQVVRHEGDP-----DFEDGDDADFPMM-DEGRQPLSM	
 361		
SEQ ID NO:2	-----	420
SEQ ID NO:4	KIPLPSSQINPYRMIIIRLVVLCCFFHYRVMHPVPDAFALWLISVICEIWFAMSWILDQ	
SEQ ID NO:6	-----	
SEQ ID NO:8	KVPISSSRINPYRMIIIVIRLVVLGFFFHYRVMHPAKDAFALWLISVICEIWFAMSWILDQ	
SEQ ID NO:10	KIPLPSSQINPYRMIIIRLVVLGFFFHYRVMHPVNDAFALWLISVICEIWFAMSWILDQ	
SEQ ID NO:12	-----	
SEQ ID NO:14	KVPIASSKINPYRMIVARLVLIAFLRLYRMLNPVHDALGLWLTSIICEIWFAFSWILDQ	
SEQ ID NO:16	-----	
SEQ ID NO:18	-----LHPVNDAYGLWLTSVICEIWFAVSWIMDQ	
SEQ ID NO:20	-----	
SEQ ID NO:22	KVPISSSRINPYRMIIIVIRLVVLGFFFHYRVMHPAKDAFALWLISVICEIWFAMSCILDQ	
SEQ ID NO:23	VVPIPSSRLTPYRVIIILRLIILCFFLQYRTTHPVKNAYPLWLTSVICEIWFAFSWLLDQ	
SEQ ID NO:24	KLPIRSSRINPYRMLILCRLAILGLFFHYRILHPVNDAYGLWLTSVICEIWFAVSWILDQ	
SEQ ID NO:25	-----	
SEQ ID NO:26	RIPIPAAIISPYRLLIVIRFVVLCCFFLTWRIRNPNEAIWLWLMIIICELWFGFSWILDQ	
SEQ ID NO:27	KVPIASSKINPYRMIVARLVLIAVFLRLYRLLNPVHDALGLWLTSVICEIWFAVSWILDQ	
SEQ ID NO:28	-----	
SEQ ID NO:29	KIPIKSSKINPYRMLIVRLVILGLFFHYRILHPVKDAYALWLISVICEIWFAVSWILDQ	
 421		
SEQ ID NO:2	-----	480
SEQ ID NO:4	FPKWFPIERETYLDRLSLRFDKEGHPS-----QLAPVDFFVSTVDPLKEPPLVTANTVLS	
SEQ ID NO:6	-----	
SEQ ID NO:8	FPKWLPIERETYLDRLSLRFDKEGQPS-----QLAPIDFFVSTVDPTKEPPLVTANTVLS	
SEQ ID NO:10	FPKWFPIERETYLDRLSLRFDKEGQPS-----QLAPIDFFVSTVDPLKEPPLVTTNTVLS	
SEQ ID NO:12	-----	
SEQ ID NO:14	FPKWFPIERETYLDRLSIRYEREGERPN-----MLAPVDVFVSTVDPMKEPPLVTANTVLS	
SEQ ID NO:16	-----	
SEQ ID NO:18	FPKWYPIQRETYLDRLSLRYEKEGKPS-----ELSSVDVFVSTVDPMKEPPLITANTVLS	
SEQ ID NO:20	-----	
SEQ ID NO:22	FPKWFPIERETYLDRLSLRFDKEGQPS-----QLAPIDFFVSTVDPTKEPPLVTANTVLS	
SEQ ID NO:23	FPKWYPINRETYLDRLAIRYDRDGEPS-----QLVPVDVFVSTVDPLKEPPLVTANTVLS	
SEQ ID NO:24	FPKWYPIERETYLDRLSLRYEKEGKPS-----GLAPVDVFVSTVDPLKEPPLITANTVLS	
SEQ ID NO:25	-----	
SEQ ID NO:26	IPLKLCPINRSTDLEVLRDKFDMPSPSNPTGRSDLPGIDLFVSTADPEKEPPLVTANTILS	
SEQ ID NO:27	FPKWFPIERETYLDRLSLRYEREGERPN-----MLAPVDVFVSTVDPLKEPPLVTSNTVLS	
SEQ ID NO:28	-----	
SEQ ID NO:29	FPKWYPIERETYLDRLSLRYEKEGKPS-----GLSPVDVFVSTVDPLKEPPLITANTVLS	
 481		
SEQ ID NO:2	-----	540
SEQ ID NO:4	ILSVDPVDKVSCYVSDGAAMLTFEALSETSEFAKKWVPFCRYSLEPRAPEWYFQQ--	
SEQ ID NO:6	-----H-----	
SEQ ID NO:8	ILSVDPVVEKVSCYVSDGAAMLTFEALSETSEFAKKWVPFSKKFNIEPRAPEWYFQQ--	
SEQ ID NO:10	ILSVDPVDKVSCYVSDGAAMLTFEALSETSEFAKKWVPFCRYSLEPRAPEWYFQQ--	
SEQ ID NO:12	ILAAGYPAGKVTCKYISDDAGAEVTRNAVVEARFAALWVSFCRKHGVEPRNLEAYFNAGE	
SEQ ID NO:14	ILAMDYPVDKISCYISDDGASMCTFESLSETAEFARKWVPFCKKFSIEPRAPEMFSE--	
SEQ ID NO:16	-----	
SEQ ID NO:18	ILAVIDYPVDKVACYVSDGAAMLTFEALSETSEFARRWVPFCCKYNIIEPRAPEWYFGQ--	

Figure 1 (cont'd.)

SEQ ID NO:20	-----	
SEQ ID NO:22	ILSVDYPVEKVSCYVSDDGAAMLTFEALSETSEFAKKWPFSKKENIEPRAPEWYFQQ--	
SEQ ID NO:23	ILSVDYPVDKVACYVSDDGSAMLTFESLSETAEFAKKWPFCKKFNIEPRAPEFYFAQ--	
SEQ ID NO:24	ILAVIDYPVDKVACYVSDDGAAMLTFEALSDTAEFARKWPFCKKFNIEPRAPEWYFSQ--	
SEQ ID NO:25	-----RRWPFCKKHNVEPRAPEFYFNE--	
SEQ ID NO:26	ILAVIDYPVEKVSCYLSDDGGALLSFEAMAEAAASFADLWVPFCRKHNIEPRNPDSYFSL--	
SEQ ID NO:27	ILAMDYPVEKISCYVSDDGASMLTFESLSETAEFARKWPFCKKFSIEPRAPEMYFTL--	
SEQ ID NO:28	---DYPVEKVSCYVSDDGAAMLTFEALSETSEFARKWPFCKKYNNIEPRAPEWYFAQ--	
SEQ ID NO:29	ILAVIDYPVDKVACYVSDDGAAMLTFEALSETAEFARKWPFCKKYCIEPRAPEWYFCH--	
 541		
SEQ ID NO:2	-----	600
SEQ ID NO:4	KIDYLKDVKAPNFVRERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:6	-----	
SEQ ID NO:8	KIDYLKDVKVAASFVRERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:10	KIDYLKDVKVAANFVRERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:12	GGGGKAKVVARGSY-RGMAWPELVRDRRRVRREYEMRLRIDALQAADARRR-----	
SEQ ID NO:14	KIDYLKDVKQPTFVKERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:16	-----AKAQ-----	
SEQ ID NO:18	KMDYLNKVKHPAFVRERRAMKRDYEEFKVRINSLVATAQ-----	
SEQ ID NO:20	-----	
SEQ ID NO:22	KIDYLKDVKVAASFVRERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:23	KIDYLKDQPSFVKERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:24	KMDYLNKVKHPAFVRERRAMKRDYEEFKVKINALVATAQ-----	
SEQ ID NO:25	KIDYLKDVKHPSFVKERRAMKREYEEFKVRINALVAKAQ-----	
SEQ ID NO:26	KIDPTKNKSRIDFVKDRRKIKREYDEFKVRINGLPDSIRRRSDAFNAREE-----	
SEQ ID NO:27	KVDYLNQDKVHPTFVKERRAMKREYEEFKVRINAQVAKAS-----	
SEQ ID NO:28	KIDYLKDVKQTSFVKERRAMKREYEEFKVRVNGLVAKAQ-----	
SEQ ID NO:29	KMDYLNKVKHPAFVRERRAMKRDYEEFKVKINALVATAQ-----	
 601		
SEQ ID NO:2	-----	660
SEQ ID NO:4	KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL-----	
SEQ ID NO:6	-----	
SEQ ID NO:8	KVPEEGWTMQDGSPWP-----NNVRDHPGMIQVFL-----	
SEQ ID NO:10	KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL-----	
SEQ ID NO:12	-----	
SEQ ID NO:14	KVPOGGWIMQDGTWPWG-----RRGAADDHAGVVQVLIDFA-----	
SEQ ID NO:16	KMPEEGWTMQDGTWPWG-----NNTRDHPGMIQVFL-----	
SEQ ID NO:18	KVPEDGWTMQDGTWPWG-----NNVRDHPGMIQVFL-----	
SEQ ID NO:20	-----	
SEQ ID NO:22	KVPEEGWTMQDGSPWP-----	
SEQ ID NO:23	KIPEEGWTMQDGTWPWG-----NNTRDHPGMIQVFL-----	
SEQ ID NO:24	KVPEEGWTMQDGTWPWG-----NNVRDHPGMIQVFL-----	
SEQ ID NO:25	KKPEEGWVMQDGTWPWG-----NNTRDHPGMIQVFL-----	
SEQ ID NO:26	MKALKQMRESGGDPTEPVKVKPATW-MADGTHWPGTWAASTREHSKGDHAGILQVMLKPP-----	
SEQ ID NO:27	KVPLEGWIMQDGTWPWG-----NNTRDHPGMIQVFL-----	
SEQ ID NO:28	KVPEEGWIMQDGTWPWG-----NNTRDHPGMIQVFL-----	
SEQ ID NO:29	KVPEDGWTMQDGTWPWG-----NSVRDHPGMIQVFL-----	
 661		
SEQ ID NO:2	-----	720
SEQ ID NO:4	G-QSGGHDV-----GNELPRLVYVSREKRPGYNHHKKAGAMNALVRSAVLNA-----	
SEQ ID NO:6	-----	
SEQ ID NO:8	G-QSGGRDV-----GNELPRLVYVSREKRPGYNHHKKAGAMNALVRSAVLNA-----	
SEQ ID NO:10	G-QSGGLDCE-----GNELPRLVYVSREKRPGYNHHKKAGAMNALVRSAVLNA-----	
SEQ ID NO:12	GSVPQLGVANGSKLIDVASDVCLPALVYVCREKRRGHAAHRKAGAMNA-----	

Figure 1 (cont'd.)

SEQ ID NO:14	-----G-SGGGLDTE-----GNQLPRLVYVSREKRPQFQHHKKAGAMNALVRSAVLTNA	
SEQ ID NO:16	-----G-HSGGLDTD-----GNELPRLVYVSREKRPQFQHHKKAGAMNALIRVSAVLTNG	
SEQ ID NO:18	-----G-QDGVRDVE-----GNELPRLVYVSREKRPQFDHHKKAGAMNALVRASAIITNA	
SEQ ID NO:20	-----	
SEQ ID NO:22	-----	
SEQ ID NO:23	-----G-HSGGLDTD-----GNELPRLIYVSREKRPQFQHHKKAGAMNALIRVSAVLTNG	
SEQ ID NO:24	-----G-HSGVRDTD-----GNELPRLVYVSREKRPQFDHHKKAGAMNSLIRVSAVLSNA	
SEQ ID NO:25	-----G-SAGALDVD-----GKEPLPRLVYVSREKRPQYQHHKKAGAENALVRVSAVLTNA	
SEQ ID NO:26	SSDPLIG-NSDDKV1DFSDTDTRLPMFVYVSREKRPQYDHNKKAGAMNALVRVASAILSNG	
SEQ ID NO:27	-----G-HSGGFDFVE-----GHEPLPRLVYVSREKRPQFQHHKKAGAMNALVRVAGVLTNA	
SEQ ID NO:28	-----G-QSGGLDAE-----GNELPRLVYVSREKRPQFQHHKKAGAMNALVRVSAVLTNG	
SEQ ID NO:29	-----G-SDGVRDVE-----NNELPRLVYVSREKRPQFDHHKKAGAMNSLIRVGVLNSA	
 721		
SEQ ID NO:2	-----	
SEQ ID NO:4	PYLLNLDCDHYINNSKAIKEAMCFMMDPLLGKK-----VCYVQFPQRFDGIDRHDRYAN	
SEQ ID NO:6	-----	
SEQ ID NO:8	AYLLNLDCDHYINNSKAIKEAMCFMMDPLVGKK-----VCYVQFPQRFDGIDKNDRYAN	
SEQ ID NO:10	PYLLNLDCDHYINNSKAIKEAMCFMMDPLLGKK-----VCYVQFPQRFDGIDRHDRYAN	
SEQ ID NO:12	PFILNLDCDHYYVNNSQLRAGICFMIERGGGAAEDAGAVAFVQFPQRVDGVDPGDRYAN	
SEQ ID NO:14	PFMLNLDCDHYVNNSKAAREAMCFLMDPQTGKK-----VCYVQFPQRFDGIDTHDRYAN	
SEQ ID NO:16	AYLLNVDCDHYNNSKALKEAMCFMMDPVLGKK-----TCYVQFPQRFDGIDLHDRYAN	
SEQ ID NO:18	PYLLNVDCDHYINNSKALREAMCFMMDPQLGKK-----VCYVQFPQRFDGIDRHDYSN	
SEQ ID NO:20	-----EAMCFLMDPNLGPO-----VCYVQFPQRFDGIDRNDRYAN	
SEQ ID NO:22	-----	
SEQ ID NO:23	AYLLNVDCDHYFNNNSKAIKEAMCFMMDPAIGKK-----CCYVQFPQRFDGIDLHDRYAN	
SEQ ID NO:24	PYLLNVDCDHYINNSKAIRESMCFCMMDPQS GKK-----VCYVQFPQRFDGIDRHDYSN	
SEQ ID NO:25	PFILNLDCDHYINNSKAMREAMCFLMDPQFGKK-----LCYVQFPQRFDGIDRHDRYAN	
SEQ ID NO:26	PFILNLDCDHYIYNCKAVREGMCFCMMDRG-GED-----ICYIQFPQRFEGIDPSDRYAN	
SEQ ID NO:27	PFMLNLDCDHYVNNNSKAVREAMCFLMDPQ1GKK-----VCYVQFPQRFDGIDTNDRYAN	
SEQ ID NO:28	AFLNLDCDHYINNSKALREAMCFLMDPNLGKQ-----VCYVQFPQRFDGIDRNDRYAN	
SEQ ID NO:29	PYLLNVDCDHYINNSKALREAMCFMMDPQS GKK-----ICYVQFPQRFDGIDRHDYSN	
 781		
SEQ ID NO:2	-----	840
SEQ ID NO:4	RNVVFFDINMKGLDGIQGPIYVGTGCVFRQALYGYDAP---KTKKPPSRTCNCWPWKCI	
SEQ ID NO:6	-----	
SEQ ID NO:8	RNVVFFDINMKGLDGIQGPIYVGTGCVFRQALYGYDAP---KTKKPPSRTCNCWPWKCL	
SEQ ID NO:10	RNVVFFDINMKGLDGIQGPIYVGTGCVFRQALYGYDAP---KTKKPPSRTCNCWPWKCF	
SEQ ID NO:12	HNRVLFDCTELGLDGLQGPIYVGTGCLFRRALYSVDLPR-----	
SEQ ID NO:14	RNTVFFDINMKGLDGIQGPVYVGTGCVFRQALYGYDAP---RPKMVSCDC-----	
SEQ ID NO:16	RNIVFFDINMKQDGQVGPVYVGTGCCFNQRQALYGYDPVLTEEDLE-----PNIIV	
SEQ ID NO:18	RNVVFFDINMKGLDGIQGPIYVGTGCVFRQALYGYDAP---AKKKPPSKTCNCWPWKCC	
SEQ ID NO:20	RNTVFFDINLRGLDGIQGPVYVGTGCVFNRTAIYGEPPIKAK---K-----PGFLA	
SEQ ID NO:22	-----	
SEQ ID NO:23	RNIVFFDINMKGLDGIQGPVYVGTGCCFNQRQALYGYDPVLTEEDLE-----PNIIV	
SEQ ID NO:24	RNVVFFDINMKGLDGIQGPIYVGTGCVFRQALYGYDAP---KKKKPPGKTCNCWPWKCC	
SEQ ID NO:25	RNVVFFDINMLGLDGLQGPVYVGTGCVFRQALYGYDPVVSEKRPK---MTCDCWPSWCC	
SEQ ID NO:26	NNTVFFDGNMRALDGQVGPVYVGTGTMFRRALYGYDPP-----	
SEQ ID NO:27	RNTVFFDINMKGLDGIQGPVYVGTGCVFKRQALYGYEPPKGPK---RPKMISC CGC	
SEQ ID NO:28	RNTVFFDINLRGLDGIQGPVYVGTGCVFNRTALYGYEPPLKPKHRK---TGILS	
SEQ ID NO:29	RNVVFFDINMKGLDGLQGPIYVGTGCVFRQALYGYDAP---KKKKGPRKTCNCWPWKCL	

Figure 1 (cont'd.)

841		900
SEQ ID NO:2	-----	-----
SEQ ID NO:4	CCCCFGNRKTKKKTKTSKP-----	KFEKIKKLF-KKKEQAPAYALGEIDEA--APG-
SEQ ID NO:6	-----	-----
SEQ ID NO:8	SCCC---SRNKNKKKTTKP-----	KTEKKRLLFFKKAENPSPAYALGEIDEA--APG-
SEQ ID NO:10	CCCCFGNRKQKK---TTKP-----	KTEKKRLLFFKKEENQSPAYALGEIDEA--APG-
SEQ ID NO:12	-----	-----
SEQ ID NO:14	-CPCFGSRKKYKE-----	KNDANGEAASLKG
SEQ ID NO:16	-KSCCGSRKKGKGNNK-----	YS-DKKKAMGR--TESTVPIFNMEDIEEGVEG--Y
SEQ ID NO:18	LCC--GSRKKKN---ANS-----	KKEKKRKV--KHSEASKQIHALENIEAGN--EG-
SEQ ID NO:20	-SLCXG-KKKASKSKKR-----	SSDKKSNKH--VDSSVPVNLEDIEEGVEGAGF
SEQ ID NO:22	-----	-----
SEQ ID NO:23	-KSCCGSRKKGKSS-KK-----	YNYEKRRGINR--SDSNAPLFNMEDIDEFGFEG--Y
SEQ ID NO:24	LCC--GLRKKSK---T-----	KAKDKKT--NTKETSKQIHALENVDEGVIVPV-
SEQ ID NO:25	-CCCGGSRKKSKKGEKKGLLGGGLLYGKKKKMMGKNVVKKGSAPVFDLEEIEEGLEG--Y	-----
SEQ ID NO:26	-----NPDKLLEKESETEALTTSDFDPLDVTQLPKRFGNSTLL-----	AESIPI
SEQ ID NO:27	-CPCFGRRRKNN-----	FSKNDMNGDVAALGG
SEQ ID NO:28	-SLCGGSRKKSSKSSKK-----	GSDKKKSGKH--VDSTVPVNLEDIEEGVEGAGF
SEQ ID NO:29	LCF--GSRKNRK---AKT-----	VAADKKK--KNREASKQIHALENIEEGRGHKV-
 901		960
SEQ ID NO:2	-----	-----
SEQ ID NO:4	AENEKAGIVNQQKLEKKFGQSSVFVASTLLENGGTLSASPASLLKEAIHVISCGYEDKT	-----
SEQ ID NO:6	-----	ET
SEQ ID NO:8	ADIEKAGIVNQQKLEKKFGQSSVFVASTLLENGGTLSASPASLLKEAIHVISCGYEDKT	-----
SEQ ID NO:10	AENEKAGIVNQQKLEKKFGQSSVFVASTLLENGGTLSASPASLLKEAIHVISCGYEDKT	-----
SEQ ID NO:12	-----	-----
SEQ ID NO:14	MDDDKEVLMQSQMNFEEKFGQSSI FVTSTLMEEGGVPPSSPAALLKEAIHVISCGYEDKT	-----
SEQ ID NO:16	DD-ERTLLMSQKSLEKRGFGQSPVFIAATFMEQGGI PPTTNPATLLKEAIHVISCGYEDKT	-----
SEQ ID NO:18	TNNEKTSNLQTKEKRGFGQSPVFVASTLLEDDGGVPHGVSPASLLKEAIQVISCGYEDKT	-----
SEQ ID NO:20	DD-EKSVLMSQMSLEKRGFGQSAFVASTLMEYGGVPOSSTPESLLKEAIHVISCGYEDKS	-----
SEQ ID NO:22	-----	-----
SEQ ID NO:23	DD-ERSILMSQRSVEKRGFGQSPVFIAATFMEQGGI PPTTNPATLLKEAIHVISCGYEDKT	-----
SEQ ID NO:24	SNVEKRSEATQLKLEKKFGQSPVFVASAVLQNGGVPRNASPACLLREAIQVISCGYEDKT	-----
SEQ ID NO:25	EELEKSTLMSQKNFEKRGFGQSPVFIASTLMEENGGLPEGTNSTSLIKEAIHVISCGYEEKT	-----
SEQ ID NO:26	AEFQGRPLADHPAV--KYGRPP---GALR---VPRDPLDATTVAESVSVISCWYEDKT	-----
SEQ ID NO:27	AEGDKEHLMFEMNFETKTFGQSSI FVTSTLMEEGGVPPSSPAVLLKEAIHVISCGYEDKT	-----
SEQ ID NO:28	DD-EKSLLMSQMSLEKRGFGQSAFVASTLMEYGGVPOSSTPESLLKEAIHVISCGYEDKT	-----
SEQ ID NO:29	LNVEQSTEAMQMKLQKKYQGSPVFVASARLENGGMARNASPACLLKEAIQVISRGYEDKT	-----
 961		1020
SEQ ID NO:2	-----HEDITGFKMHARGWISIYCMPPRPFCKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:4	GWGKDIGHWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:6	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:8	DWGKEIGWIYGSITEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:10	DWGKEIGWIYGSITEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:12	-----WRP-----RRSL	-----
SEQ ID NO:14	EWGLELGWIYGSITEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:16	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:18	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:20	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:22	-----	-----
SEQ ID NO:23	EWGKEIGWIYGSVTEDILTGFKMHARGWISIYCNPPRPAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:24	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:25	EWGKEIGWIYGSVTEDILTGFKMHCHGWRISIYCIPKRAFKGSAPI NLSDRLNQVLRWAL	-----
SEQ ID NO:26	EWGDRVGIYGSVTEDVVTGYRMHNRGWRSVYCITKRDSSFRGSAPI NLTDRLNQVLRWAT	-----

Figure 1 (cont'd.)

SEQ ID NO:27	EWGTELGIYGSITEDILTGFKMHCRGWRHSIYCMPCRPAFKGSAPINLSDRLNQVLRWAL	
SEQ ID NO:28	DWGSEIGWIYGSVTEDILTGFKMHARGWRHSIYCMPCRPAFKGSAPINLSDRLNQVLRWAL	
SEQ ID NO:29	EWGKEIGWIYGSVTEDILTGSKMHSHGWRHVCTPKLAALKGSAPINLSDRLHQVLRWAL	
 1021		
SEQ ID NO:2	GSVEILFSRHCPIWNYGG-RLKLLERMAYINTIVYPITSPLIAYCVLPAICLLTNKFI	1080
SEQ ID NO:4	GSIEIFFSNHCPLWYGYGGG-LKFLERFSYINSIVYPWTSIPLLAYCTLPACCLLTGKFI	
SEQ ID NO:6	GSVEIFMSRHCPLWYAYGG-RLKWLRFAYNTIVYPFTSIPLLAYCTIPAVCCLLTGKFI	
SEQ ID NO:8	GSVEIFFSKHCPLWYGYGGG-LKFLERFSYINSIVYPWTSIPLLAYCTLPACCLLTGKFI	
SEQ ID NO:10	GSIEIFFSNHCPLWYGYGGG-LKFLERFSYINSIVYPWTSIPLLAYCTLPACCLLTGKFI	
SEQ ID NO:12	G-----CRL-----	
SEQ ID NO:14	GSIEIFFSHHCPLWYGFKEKKLKWLERFAYANTTVYPFTSIPLVAYCILPAVCCLTDKFI	
SEQ ID NO:16	GSIEIFLSRHCPLWYGYNG-KLKPLMRLAYINTIVYPFTSIPLLAYCTLPACCLLTGKFI	
SEQ ID NO:18	GSVEIFFSRHCPIWYGYGGG-LKLLERFSYINSVVYPWTSPLLLVYCTLPACCLLTGKFI	
SEQ ID NO:20	GSVEILFSRHCPLWYGYGG-RLKFLERFAYINTTIYPLTSLPLLVYCILPAICCLLTGKFI	
SEQ ID NO:22	-----	
SEQ ID NO:23	GSIEILLSRHCPIWGYHG-RLRLLERIAYINTIVYPITSIPLLAYCILPAFCCLITDRFI	
SEQ ID NO:24	GSVEIFLSRHCPIWGYGGG-LKWLRFAYNTIVYPFTSIPLLAYCILPAVCCLLTGKFI	
SEQ ID NO:25	GSVEIFLSRHCPLWYGYGG-KLKWLRFAYNTIVYPFTSIPLLAYCTIPAVCCLLTGKFI	
SEQ ID NO:26	GSVEIFFSRNNNAI---LASKRLKFLQRLAYLNVGIYPPFTSFLFLYLICFLPAFSLFSGQFI	
SEQ ID NO:27	GSVEIFFSRHSPLWYGYKGGKLKWLERFAYANTTIYPTSIPLLAYCILPAICCLTDKFI	
SEQ ID NO:28	GSVEILFSRHCPIWYGYSR-RLKWLRFAYVNTTIYPTAIPLLMYCTLPAVCCLTNKFI	
SEQ ID NO:29	GSVEIFLSRHCPIWYGYGGG-LKWLRLSYINSVVYPWTSPLIVYCSLPAICCLLTGKFI	
 1081		
SEQ ID NO:2	IPEISNYAGMFILMFASIFATGILELRWSGVGIEDWWRNEQFWVIGGTS AHLFAVFQGL	1140
SEQ ID NO:4	TPELNNVASLWFMSLFICIFATSILEMRWSGVGIDDDWWRNEQFWVIGGVSSHLFAVFQGL	
SEQ ID NO:6	IPTELNNAISIWFIALFLSIIATSVLELRWSGVSIEDWWRNEQFWVIGGVSAHLSAHLFQGL	
SEQ ID NO:8	TPELTNVASIWFMALFICISVTGILEMRWSGVAIADDWWRNEQFWVIGGVSAHLSAHLFQGL	
SEQ ID NO:10	TPELNNVASLWFMSLFICIFATSILEMRWSGVGIDDDWWRNEQFWVIGGVSSHLFAVFQGL	
SEQ ID NO:12	-----	
SEQ ID NO:14	MPPISTFAGLYFVALFSSIIATGILELKWSGVSI EEWWRNEQFWVIGGVSAHLSAHLFQGL	
SEQ ID NO:16	IPEISNFASMWFILLFVSIFTTSILELRWSGVSIEDWWRNEQFWVIGGTS AHLFAVFQGL	
SEQ ID NO:18	VPEISNYASLVMALFISIAATGILEMQWGGVSIDDDWWRNEQFWVIGGVSSHLFALFQGL	
SEQ ID NO:20	MPEISNLASIWFIALFLSIFATGILEMRWSGVGIDEWWRNEQFWVIGGISAHLSAHLFQGL	
SEQ ID NO:22	-----	
SEQ ID NO:23	IPEISNYASIWFILLFISIAVTGILELRWSGVSIEDWWRNEQFWVIGGTS AHLFAVFQGL	
SEQ ID NO:24	VPEISNYAGILFMLMFISIAVTGILEMQWGGVGIDDDWWRNEQFWVIGGASSHLFALFQGL	
SEQ ID NO:25	IPTLSNLTSVWFLALFLSIIATGVLELRWSGVSIQDWWRNEQFWVIGGVSAHLSAHLFQGL	
SEQ ID NO:26	VRTLSISFLVYLLMITICLIGLAVLEVKGWVGIGLEEEWWRNEQWWLISGTSSHLYAVVQGV	
SEQ ID NO:27	MPPISTFASLFFISLFMSIIVTGILELRWSGVSI EEWWRNEQFWVIGGISAHLSAHLFQGL	
SEQ ID NO:28	IPQISNLASIWFISLFISIFATGILKMKWNGVGIDQWWRNEQFWVIGGVSAHLSAHLFQGL	
SEQ ID NO:29	VPEISNYASILFMALFSSIAITGILEMQWKGVGIDDDWWRNEQFWVIGGVSAHLSAHLFQGL	
 1141		
SEQ ID NO:2	LKVLAGIDTNFTVTSKANDEDGD--FAELYVFKWTSLLIPPTTVLVINLVGMVAGISYAI	1200
SEQ ID NO:4	LKVIAGVDTSTVTSKGGDD--EE-FSELYTFKWTTLIIPPTTLLLNFIGVVAGISNAI	
SEQ ID NO:6	LKVLGGVDTSTVTSKAAGDEADA-FGDLYLFKWTTLVPPTTLIIINMVGVAGVSDAV	
SEQ ID NO:8	LKVFAGIDTSFTVTSKAGDD--EE-FSELYTFKWTTLIIPPTTLLLNFIGVVAGISNAI	
SEQ ID NO:10	LKVIAGVDTSTVTSKGGDD--EE-FSELYTFKWTTLIIPPTTLLLNFIGVVAGVSNAI	
SEQ ID NO:12	-----	
SEQ ID NO:14	LKVLAGIDTNFTVTSKATDDE-E--FGELYTFKWTTLIIPPTTLLIINIVGVVAGISDAI	
SEQ ID NO:16	LKVLAGIDTNFTVTSKASDEDGD--FAELYVFKWTSLLIPPTTVLIVNLVGVAGVSYAI	
SEQ ID NO:18	LKVLAGVNTNFTVTSKAADD--GE-FSELYIFKWTSLIIPPTTLLIMNIVGVVVGISDAI	
SEQ ID NO:20	LKVLAGIDTNFTVTSKANDEEGD--FAELYMFKWTTLIIPPTTLLIINMVGVVAGTSYAI	
SEQ ID NO:22	-----	
SEQ ID NO:23	LKVLAGIDTNFTVTSKATDEDGD--FAELYIFKWTALLIPPTTVLLVNLIGIVAGVSYAV	

Figure 1 (cont'd.)

SEQ ID NO:24	LKVLAGVNTNFTVTSKAADD--GA-FSELYIFKWTTLIIPPTTLLIINIIGVIVGVSDAI	
SEQ ID NO:25	LKVLAGVDTNFTVTKAADDTE---FGELYLFKWTTLIIPPTTLLIILNMVGVVAGVSDAI	
SEQ ID NO:26	LKVIAGIEISFTLTTKSGGDDNEDIYADLYIVWKSSLMIPPIVIAVNIIIAIVVAFIRTI	
SEQ ID NO:27	LKILAGIDTNFTVTSKATDDD-D--FGELYAFKWTTLIIPPTTVLIINIVGVVAGISDAI	
SEQ ID NO:28	LKVLAGIDTNFTVTSKASDEDGD--FAELYMFKWTTLIIPPTTLLIINLVGVVAGISYVI	
SEQ ID NO:29	LKVLAGVDTNFTVTSKAADD--GE-FSDLYLFKWTSLLIIPPTMILLINVIGVIVGVSDAI	
 1201		
SEQ ID NO:2	NNGYQSWGPLFGKLFFSIWVILHLYPFLKGKLMGKQNRTPTIVIVWSILLASIFSLLWVKI	1260
SEQ ID NO:4	NNGYESWGPLFGKLFFAFWVIVHLYPFLKGKLMGRQNRTPTIVIVWSILLASIFSLLWVRI	
SEQ ID NO:6	NNGYGSWGPLFGKLFFSFWVIVHLYPFLKGKLMGRQNRTPTIVVLSILLASIFSLLWVRI	
SEQ ID NO:8	NNGYESWGPLFGKLFFAFWVIVHLYPFLKGKLMGRQNRTPTIVIVWSILLASIFSLLWVRV	
SEQ ID NO:10	NNGYESWGPLFGKLFFAFWVIVHLYPFLKGKLMGRQNRTPTIVIVWSILLASIFSLLWVRI	
SEQ ID NO:12	-----LGEDER-----LWSRM	
SEQ ID NO:14	NNGYQSWGPLFGKLFFSFWVIVHLYPFLKGKLMGRQNRTPTIVVIWSVLLASIFSLLWVRI	
SEQ ID NO:16	NNGYQSWGPLFGKLFFAFWVIVAHLYPFLKGKLLGRQNRTPTIVIVWSVLLASIFSLLWVRI	
SEQ ID NO:18	NNGYDSWGPLFGRLFFALWVILHLYPFLKGKLLGKQDRMPTIILVWSILLASILTLMWVRI	
SEQ ID NO:20	NNGYQSWGPLFGKLFFAFWVIVHLYPFLKGKLMGRQNRTPTIVIVWAULLASIFSLLWVRI	
SEQ ID NO:22	-----	
SEQ ID NO:23	NNGYQSWGPLFGKLFFALWVIAHLYPFLKGKLLGRQNRTPTIVIVWSVLLASIFSLLWVRI	
SEQ ID NO:24	SNGYDSWGPLFGRLFFALWVIVHLYPFLKGMLGKQDKMPTIIVVWSILLASILTLLWVRV	
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SEQ ID NO:12	KQMVLISGPR-----	
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Ala Ile Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu Ile Ser Asn  
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Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly Leu Met  
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Pro Gly Ile Trp Arg Ser Gly Ser Ala Arg Gly Met Glu Ala Ser Ala  
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Gly Leu Val Ala Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg  
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Arg Asp Gly Glu Pro Gly Pro Lys Pro Met Asp Gln Arg Asn Gly Gln  
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Ser Val Glu Ile Phe Met Ser Arg His Cys Pro Leu Trp Tyr Ala Tyr  
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&lt;210&gt; 10

&lt;211&gt; 1086

&lt;212&gt; PRT

&lt;213&gt; Zea mays

&lt;400&gt; 10

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Leu	Val	Val	Ile	Arg	Arg	Asp	Gly	Asp	Pro	Gly	Pro	Lys	Pro	Pro	Arg
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Glu	Gln	Asn	Gly	Gln	Val	Cys	Gln	Ile	Cys	Gly	Asp	Asp	Val	Gly	Leu
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Ala	Pro	Gly	Gly	Asp	Pro	Phe	Val	Ala	Cys	Asn	Glu	Cys	Ala	Phe	Pro
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Val	Cys	Arg	Asp	Cys	Tyr	Glu	Tyr	Glu	Arg	Arg	Glu	Gly	Thr	Gln	Asn
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Cys	Pro	Gln	Cys	Lys	Thr	Arg	Tyr	Lys	Arg	Leu	Lys	Gly	Cys	Gln	Arg
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Val	Thr	Gly	Asp	Glu	Glu	Asp	Gly	Val	Asp	Asp	Leu	Asp	Asn	Glu	
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Phe	Asn	Trp	Asp	Gly	His	Asp	Ser	Gln	Ser	Val	Ala	Glu	Ser	Met	Leu
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Tyr	Gly	His	Met	Ser	Tyr	Gly	Arg	Gly	Asp	Pro	Asn	Gly	Ala	Pro	
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Gln	Ala	Phe	Gln	Leu	Asn	Pro	Asn	Val	Pro	Leu	Leu	Thr	Asn	Gly	Gln
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 Ser Leu Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala  
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 Lys Gln Arg Gln Glu Arg Met His Gln Thr Gly Asn Asp Gly Gly  
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 Asp Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln  
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 Gln Leu Ser Arg Lys Ile Pro Leu Pro Ser Ser Gln Ile Asn Pro Tyr  
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 Arg Met Ile Ile Ile Ile Arg Leu Val Val Leu Gly Phe Phe Phe His  
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 Tyr Arg Val Met His Pro Val Asn Asp Ala Phe Ala Leu Trp Leu Ile  
                   290                  295                  300  
  
 Ser Val Ile Cys Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp Gln  
                   305                  310                  315                  320  
  
 Phe Pro Lys Trp Phe Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu  
                   325                  330                  335  
  
 Ser Leu Arg Phe Asp Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile  
                   340                  345                  350  
  
 Asp Phe Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val  
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 Thr Thr Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp  
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 Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe  
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 Glu Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe  
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 Cys Lys Arg Tyr Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln  
                   420                  425                  430  
  
 Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Ala Asn Phe Val Arg  
                   435                  440                  445  
  
 Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile  
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 Asn Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr  
                   465                  470                  475                  480

Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro  
                   485                  490                  495  
 Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly Leu Asp Cys Glu  
                   500                  505                  510  
 Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro  
                   515                  520                  525  
 Gly Tyr Asn His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg  
                   530                  535                  540  
 Val Ser Ala Val Leu Thr Asn Ala Pro Tyr Leu Leu Asn Leu Asp Cys  
                   545                  550                  555                  560  
 Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe  
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 Met Met Asp Pro Leu Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro  
                   580                  585                  590  
 Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Arg Tyr Ala Asn Arg Asn  
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 Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly  
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 Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr  
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 Gly Tyr Asp Ala Pro Lys Thr Lys Lys Pro Pro Ser Arg Thr Cys Asn  
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 Cys Trp Pro Lys Trp Cys Phe Cys Cys Cys Phe Gly Asn Arg Lys  
                   660                  665                  670  
 Gln Lys Lys Thr Thr Lys Pro Lys Thr Glu Lys Lys Lys Leu Leu Phe  
                   675                  680                  685  
 Phe Lys Lys Glu Glu Asn Gln Ser Pro Ala Tyr Ala Leu Gly Glu Ile  
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 Gln Gln Lys Leu Glu Lys Lys Phe Gly Gln Ser Ser Val Phe Val Thr  
                   725                  730                  735  
 Ser Thr Leu Leu Glu Asn Gly Gly Thr Leu Lys Ser Ala Ser Pro Ala  
                   740                  745                  750  
 Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp  
                   755                  760                  765  
 Lys Thr Asp Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr  
                   770                  775                  780  
 Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser  
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Ile Tyr Cys Ile Pro Lys Arg Val Ala Phe Lys Gly Ser Ala Pro Leu  
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Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser  
 820 825 830

Ile Glu Ile Phe Phe Ser Asn His Cys Pro Leu Trp Tyr Gly Tyr Gly  
 835 840 845

Gly Gly Leu Lys Phe Leu Glu Arg Phe Ser Tyr Ile Asn Ser Ile Val  
 850 855 860

Tyr Pro Trp Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Leu Pro Ala  
 865 870 875 880

Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr Pro Glu Leu Asn Asn Val  
 885 890 895

Ala Ser Leu Trp Phe Met Ser Leu Phe Ile Cys Ile Phe Ala Thr Ser  
 900 905 910

Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile Asp Asp Trp Trp Arg  
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Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ser His Leu Phe Ala  
 930 935 940

Val Phe Gln Gly Leu Leu Lys Val Ile Ala Gly Val Asp Thr Ser Phe  
 945 950 955 960

Thr Val Thr Ser Lys Gly Gly Asp Asp Glu Glu Phe Ser Glu Leu Tyr  
 965 970 975

Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Leu  
 980 985 990

Leu Asn Phe Ile Gly Val Val Ala Gly Val Ser Asn Ala Ile Asn Asn  
 995 1000 1005

Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe  
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Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Val Gly Arg  
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Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu Ala  
 1045 1050 1055

Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Leu Ala Lys  
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Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly Leu Asp Cys Asn  
 1075 1080 1085

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<212> DNA  
<213> Oryza sativa

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&lt;210&gt; 12

&lt;211&gt; 341

&lt;212&gt; PRT

&lt;213&gt; Oryza sativa

&lt;400&gt; 12

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Arg	Ser	Arg	Arg	Ser	Pro	Arg	Arg	Thr	Pro	Cys	Cys	Pro	Tyr	Ile	Leu
														20	30

Ala	Ala	Gly	Tyr	Pro	Ala	Gly	Lys	Val	Thr	Cys	Tyr	Ile	Ser	Asp	Asp
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Ala	Gly	Ala	Glu	Val	Thr	Arg	Asn	Ala	Val	Val	Glu	Ala	Ala	Arg	Phe
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Ala	Ala	Leu	Trp	Val	Ser	Phe	Cys	Arg	Lys	His	Gly	Val	Glu	Pro	Arg
														65	80

Asn	Leu	Glu	Ala	Tyr	Phe	Asn	Ala	Gly	Glu	Gly	Gly	Gly	Lys	Ala	
														85	95

Lys	Val	Val	Ala	Arg	Gly	Ser	Tyr	Arg	Gly	Met	Ala	Trp	Pro	Glu	Leu
														100	110

Val	Arg	Asp	Arg	Arg	Arg	Val	Arg	Arg	Glu	Tyr	Glu	Glu	Met	Arg	Leu
														115	125

Arg	Ile	Asp	Ala	Leu	Gln	Ala	Ala	Asp	Ala	Arg	Arg	Arg	Arg	Arg	Gly
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Ala	Ala	Asp	Asp	His	Ala	Gly	Val	Val	Gln	Val	Leu	Ile	Asp	Phe	Ala
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Gly	Ser	Val	Pro	Gln	Leu	Gly	Val	Ala	Asn	Gly	Ser	Lys	Leu	Ile	Asp
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Val Ala Ser Val Asp Val Cys Leu Pro Ala Leu Val Tyr Val Cys Arg  
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Glu Lys Arg Arg Gly His Ala His His Arg Lys Ala Gly Ala Met Asn  
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Ala Pro Phe Ile Leu Asp Leu Asp Cys Asp Tyr Tyr Val Asn Asn Ser  
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Gln Ala Leu Arg Ala Gly Ile Cys Phe Met Ile Glu Arg Gly Gly Gly  
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Gly Ala Ala Glu Asp Ala Gly Ala Val Ala Phe Val Gln Phe Pro Gln  
 245 250 255

Arg Val Asp Gly Val Asp Pro Gly Asp Arg Tyr Ala Asn His Asn Arg  
 260 265 270

Val Leu Phe Asp Cys Thr Glu Leu Gly Leu Asp Gly Leu Gln Gly Pro  
 275 280 285

Ile Tyr Val Gly Thr Gly Cys Leu Phe Arg Arg Val Ala Leu Tyr Ser  
 290 295 300

Val Asp Leu Pro Arg Trp Arg Pro Arg Arg Ser Leu Gly Cys Arg Leu  
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Leu Gly Glu Asp Glu Arg Leu Trp Ser Arg Met Lys Gln Met Val Ile  
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Leu Ser Gly Pro Arg  
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<210> 13

<211> 3517

<212> DNA

<213> Glycine max

<400> 13

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Asp Gly Gln Val Cys Glu Ile Cys Gly Asp Gly Val Gly Leu Thr Val  
35 40 45

Asp Gly Asp Leu Phe Val Ala Cys Asn Glu Cys Gly Phe Pro Val Cys  
50 55 60

Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Ser His Leu Cys Pro  
 65 70 75 80  
 Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Ser Pro Arg Val Glu  
 85 90 95  
 Gly Asp Asp Asp Glu Glu Asp Val Asp Asp Ile Glu His Glu Phe Asn  
 100 105 110  
 Ile Asp Glu Gln Lys Asn Lys His Gly Gln Val Ala Glu Ala Met Leu  
 115 120 125  
 His Gly Arg Met Ser Tyr Gly Arg Gly Pro Glu Asp Asp Asn Ser  
 130 135 140  
 Gln Phe Pro Thr Pro Val Ile Ala Gly Gly Arg Ser Arg Pro Val Ser  
 145 150 155 160  
 Gly Glu Phe Pro Ile Ser Ser Asn Ala Tyr Gly Asp Gln Met Leu Ser  
 165 170 175  
 Ser Ser Leu His Lys Arg Val His Pro Tyr Pro Val Ser Glu Pro Gly  
 180 185 190  
 Ser Ala Arg Trp Asp Glu Lys Lys Xaa Asp Gly Trp Lys Asp Arg Met  
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 Val Ala Arg Leu Val Ile Leu Ala Phe Phe Leu Arg Tyr Arg Leu Met  
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 Asn Pro Val His Asp Ala Leu Gly Leu Trp Leu Thr Ser Ile Ile Cys  
 275 280 285  
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 Phe Pro Ile Asp Arg Glu Thr Tyr Leu Asp Arg Leu Ser Ile Arg Tyr  
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 Glu Arg Glu Gly Glu Pro Asn Met Leu Ala Pro Val Asp Val Phe Val  
 325 330 335  
 Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu Val Thr Ala Asn Thr  
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 Val Leu Ser Ile Leu Ala Met Asp Tyr Pro Val Asp Lys Ile Ser Cys  
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 Tyr Ile Ser Asp Asp Gly Ala Ser Met Cys Thr Phe Glu Ser Leu Ser  
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Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys Phe  
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 Tyr Leu Lys Asp Lys Val Gln Pro Thr Phe Val Lys Glu Arg Arg Ala  
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 Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val  
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 Ala Lys Ala Gln Lys Val Pro Gln Gly Gly Trp Ile Met Gln Asp Gly  
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 Thr Pro Trp Pro Gly Asn Asn Thr Lys Asp His Pro Gly Met Ile Gln  
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 Val Phe Leu Gly Ser Ser Gly Gly Leu Asp Thr Glu Gly Asn Gln Leu  
 485                   490                   495  
 Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His  
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 His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Val Ser Ala Val  
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 Leu Thr Asn Ala Pro Phe Met Leu Asn Leu Asp Cys Asp His Tyr Val  
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 Asn Asn Ser Lys Ala Ala Arg Glu Ala Met Cys Phe Leu Met Asp Pro  
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 Gln Thr Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp  
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 Gly Ile Asp Thr His Asp Arg Tyr Ala Asn Arg Asn Thr Val Phe Phe  
 580                   585                   590  
 Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr Val  
 595                   600                   605  
 Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr Gly Tyr Asn Pro  
 610                   615                   620  
 Pro Lys Gly Pro Lys Arg Pro Lys Met Val Ser Cys Asp Cys Cys Pro  
 625                   630                   635                   640  
 Cys Phe Gly Ser Arg Lys Lys Tyr Lys Glu Lys Asn Asp Ala Asn Gly  
 645                   650                   655  
 Glu Ala Ala Ser Leu Lys Gly Met Asp Asp Asp Lys Glu Val Leu Met  
 660                   665                   670  
 Ser Gln Met Asn Phe Glu Lys Lys Phe Gly Gln Ser Ser Ile Phe Val  
 675                   680                   685  
 Thr Ser Thr Leu Met Glu Glu Gly Gly Val Pro Pro Ser Ser Ser Pro  
 690                   695                   700

Ala Ala Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu  
 705 710 715 720  
 Asp Lys Thr Glu Trp Gly Leu Glu Leu Gly Trp Ile Tyr Gly Ser Ile  
 725 730 735  
 Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys Arg Gly Trp Arg  
 740 745 750  
 Ser Ile Tyr Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Thr Ala Pro  
 755 760 765  
 Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly  
 770 775 780  
 Ser Ile Glu Ile Phe Phe Ser His His Cys Pro Leu Trp Tyr Gly Phe  
 785 790 795 800  
 Lys Glu Lys Leu Lys Trp Leu Glu Arg Phe Ala Tyr Ala Asn Thr  
 805 810 815  
 Thr Val Tyr Pro Phe Thr Ser Ile Pro Leu Val Ala Tyr Cys Ile Leu  
 820 825 830  
 Pro Ala Val Cys Leu Leu Thr Asp Lys Phe Ile Met Pro Pro Ile Ser  
 835 840 845  
 Thr Phe Ala Gly Leu Tyr Phe Val Ala Leu Phe Ser Ser Ile Ile Ala  
 850 855 860  
 Thr Gly Ile Leu Glu Leu Lys Trp Ser Gly Val Ser Ile Glu Glu Trp  
 865 870 875 880  
 Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ala His Leu  
 885 890 895  
 Phe Ala Val Ile Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr  
 900 905 910  
 Asn Phe Thr Val Thr Ser Lys Ala Thr Asp Asp Glu Glu Phe Gly Glu  
 915 920 925  
 Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile  
 930 935 940  
 Leu Ile Ile Asn Ile Val Gly Val Val Ala Gly Ile Ser Asp Ala Ile  
 945 950 955 960  
 Asn Asn Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe  
 965 970 975  
 Ser Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met  
 980 985 990  
 Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Val Ile Trp Ser Val Leu  
 995 1000 1005  
 Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Val  
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<212> DNA

<213> Glycine max

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ctggatACAG	atggAAATG	gctgcctaga	cttGTTATG	tttctcgta	gaagcgacca	180
ggcttccAAC	atcacaAGAA	ggctggagCT	atGAATGCTT	tgattcgagt	ttctgtgtc	240
ttgaccaatG	gtgcataTCT	tctGAATGTG	gattgtgatC	actatTTCA	taatAGCAAA	300
gccctcaAGA	aAGCCATGTG	tttcatgtG	gatCCTGTT	ttggAAAGAA	gacatgtat	360
gttcaattTC	ctcagAGATT	tgacGGCATT	gactTGcacG	atcgatATGC	caatcgcaat	420
attgtgttCT	ttgatATCAA	catgAAAGGT	caggatGGTG	ttcaggGCC	agtctatgt	480
ggaactggtt	gttGTTCAA	taggcaAGCT	ttgtatGGTT	atgatCCTG	tttgactgag	540
gaagatttgg	aacctaACAT	tattgtAAAG	agttgttgcG	tttctagAAA	gaagggAAAG	600
ggTggcaATA	agaAGTACAG	tgacaAGAAG	aaggcGatGG	gaagaACTGA	atccactgtA	660
ccccatATTA	atatggAAAGA	catAGAGGAG	ggtgtGAAG	gttatGatGA	tGAAAGGACA	720
ctacttatGT	ctcaaAAGAG	cttggAGAAG	cgtttggcT	agtctccAGT	ttttattgt	780
gccactttCA	tggagcAGGG	tggcattCCA	ccttcaACGA	accctgcAAC	tcttcttaAG	840
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gcctatcaAA	gttgcTggA	ggattgaACC	cctgaaATAG	atggGAATGT	accctctcTG	1980
tttcttattt	ttatctacat	gttccTTACA	agaatAGTC	gtagtaatGT	tgaggtgtat	2040
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<211> 610

<212> PRT

<213> Glycine max

<400> 16

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20 25 30

Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn Glu Leu  
           35                  40                  45  
 Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln His  
           50                  55                  60  
 His Lys Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val Ser Ala Val  
   65                  70                  75                  80  
 Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His Tyr Phe  
       85                  90                  95  
 Asn Asn Ser Lys Ala Leu Lys Glu Ala Met Cys Phe Met Met Asp Pro  
       100                  105                  110  
 Val Leu Gly Lys Lys Thr Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp  
       115                  120                  125  
 Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val Phe Phe  
       130                  135                  140  
 Asp Ile Asn Met Lys Gly Gln Asp Gly Val Gln Gly Pro Val Tyr Val  
       145                  150                  155                  160  
 Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr Asp Pro  
       165                  170                  175  
 Val Leu Thr Glu Glu Asp Leu Glu Pro Asn Ile Ile Val Lys Ser Cys  
       180                  185                  190  
 Cys Gly Ser Arg Lys Lys Gly Lys Gly Asn Lys Lys Tyr Ser Asp  
       195                  200                  205  
 Lys Lys Lys Ala Met Gly Arg Thr Glu Ser Thr Val Pro Ile Phe Asn  
       210                  215                  220  
 Met Glu Asp Ile Glu Glu Gly Val Glu Gly Tyr Asp Asp Glu Arg Thr  
       225                  230                  235                  240  
 Leu Leu Met Ser Gln Lys Ser Leu Glu Lys Arg Phe Gly Gln Ser Pro  
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 Val Phe Ile Ala Ala Thr Phe Met Glu Gln Gly Gly Ile Pro Pro Ser  
       260                  265                  270  
 Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys  
       275                  280                  285  
 Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr  
       290                  295                  300  
 Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg  
       305                  310                  315                  320  
 Gly Trp Ile Ser Ile Tyr Cys Met Pro Pro Arg Pro Ala Phe Lys Gly  
       325                  330                  335  
 Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp  
       340                  345                  350

Ala Leu Gly Ser Ile Glu Ile Phe Leu Ser Arg His Cys Pro Leu Trp  
                  355                 360                 365  
 Tyr Gly Tyr Asn Gly Lys Leu Lys Pro Leu Met Arg Leu Ala Tyr Ile  
                  370                 375                 380  
 Asn Thr Ile Val Tyr Pro Phe Thr Ser Ile Pro Leu Ile Ala Tyr Cys  
                  385                 390                 395                 400  
 Thr Leu Pro Ala Phe Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu  
                  405                 410                 415  
 Ile Ser Asn Phe Ala Ser Met Trp Phe Ile Leu Leu Phe Val Ser Ile  
                  420                 425                 430  
 Phe Thr Thr Ser Ile Leu Glu Leu Arg Trp Ser Gly Val Ser Ile Glu  
                  435                 440                 445  
 Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala  
                  450                 455                 460  
 His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile  
                  465                 470                 475                 480  
 Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp  
                  485                 490                 495  
 Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro  
                  500                 505                 510  
 Thr Thr Val Leu Ile Val Asn Leu Val Gly Ile Val Ala Gly Val Ser  
                  515                 520                 525  
 Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys  
                  530                 535                 540  
 Leu Phe Phe Ala Ile Trp Val Ile Ala His Leu Tyr Pro Phe Leu Lys  
                  545                 550                 555                 560  
 Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp  
                  565                 570                 575  
 Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp  
                  580                 585                 590  
 Pro Phe Thr Ser Asp Ser Asn Lys Leu Thr Asn Gly Gln Cys Gly Ile  
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 Asn Cys  
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agcaaacact	gttctatcta	tccttgctgt	tgattatcca	tttgataaaag	ttgcatgcta	300
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tgcttaggaga	tgggttccat	ttttaagaaa	atacaatatt	gagccccggg	caccagaatg	420
gtacttttgt	cagaagatgg	actatctgaa	aaataaaagta	cacccagcat	ttgtcaggga	480
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tgtatTTTC	gatattaaaca	tgaaaggatt	ggatggata	caaggtccaa	tatatgtcgg	1020
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gatcgggggt	gtttttccc	atctatttgc	cctatttcag	ggtttactga	aggtcttggc	1980
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aaaaaaaaaaa						2890

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<211> 793  
<212> PRT  
<213> Glycine max

<400> 18

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Val Ile Cys Glu Ile Trp Phe Ala Val Ser Trp Ile Met Asp Gln Phe  
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Pro Lys Trp Tyr Pro Ile Gln Arg Glu Thr Tyr Leu Asp Arg Leu Ser  
     35                          40                          45  
 Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Glu Leu Ser Ser Val Asp  
     50                          55                          60  
 Val Phe Val Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu Ile Thr  
     65                          70                          75                          80  
 Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val Asp Lys  
     85                          90                          95  
 Val Ala Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu  
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 Ala Leu Ser Glu Thr Ser Glu Phe Ala Arg Arg Trp Val Pro Phe Cys  
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 Lys Lys Tyr Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gly Gln  
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 Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro Ala Phe Val Arg Glu  
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 Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe Lys Val Arg Ile Asn  
     165                         170                         175  
 Ser Leu Val Ala Thr Ala Gln Lys Val Pro Glu Asp Gly Trp Thr Met  
     180                         185                         190  
 Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro Gly  
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 Met Ile Gln Val Phe Leu Gly Gln Asp Gly Val Arg Asp Val Glu Gly  
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 Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly  
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 Phe Asp His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg Ala  
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 Ser Ala Ile Ile Thr Asn Ala Pro Tyr Leu Leu Asn Val Asp Cys Asp  
     260                         265                         270  
 His Tyr Ile Asn Asn Ser Lys Ala Leu Arg Glu Ala Met Cys Phe Met  
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 Met Asp Pro Gln Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln  
     290                         295                         300  
 Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr Ser Asn Arg Asn Val  
     305                         310                         315                         320  
 Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro  
     325                         330                         335  
 Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Tyr Ala Leu Tyr Gly  
     340                         345                         350

Tyr Asp Ala Pro Ala Lys Lys Lys Pro Pro Ser Lys Thr Cys Asn Cys  
 355 360 365  
 Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Ser Arg Lys Lys Lys Asn  
 370 375 380  
 Ala Asn Ser Lys Lys Glu Lys Lys Arg Lys Val Lys His Ser Glu Ala  
 385 390 395 400  
 Ser Lys Gln Ile His Ala Leu Glu Asn Ile Glu Ala Gly Asn Glu Gly  
 405 410 415  
 Thr Asn Asn Glu Lys Thr Ser Asn Leu Thr Gln Thr Lys Leu Glu Lys  
 420 425 430  
 Arg Phe Gly Gln Ser Pro Val Phe Val Ala Ser Thr Leu Leu Asp Asp  
 435 440 445  
 Gly Gly Val Pro His Gly Val Ser Pro Ala Ser Leu Leu Lys Glu Ala  
 450 455 460  
 Ile Gln Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys  
 465 470 475 480  
 Glu Val Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly  
 485 490 495  
 Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr Cys Ile Pro Lys  
 500 505 510  
 Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu  
 515 520 525  
 His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Phe Phe Ser  
 530 535 540  
 Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly Leu Lys Leu Leu  
 545 550 555 560  
 Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro Trp Thr Ser Leu  
 565 570 575  
 Pro Leu Leu Val Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly  
 580 585 590  
 Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Ser Leu Val Phe Met  
 595 600 605  
 Ala Leu Phe Ile Ser Ile Ala Ala Thr Gly Ile Leu Glu Met Gln Trp  
 610 615 620  
 Gly Gly Val Ser Ile Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val  
 625 630 635 640  
 Ile Gly Gly Val Ser Ser His Leu Phe Ala Leu Phe Gln Gly Leu Leu  
 645 650 655  
 Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val Thr Ser Lys Ala  
 660 665 670

Ala Asp Asp Gly Glu Phe Ser Glu Leu Tyr Ile Phe Lys Trp Thr Ser  
 675                    680                    685

Leu Leu Ile Pro Pro Met Thr Leu Leu Ile Met Asn Ile Val Gly Val  
 690                    695                    700

Val Val Gly Ile Ser Asp Ala Ile Asn Asn Gly Tyr Asp Ser Trp Gly  
 705                    710                    715                    720

Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val Ile Leu His Leu  
 725                    730                    735

Tyr Pro Phe Leu Lys Gly Leu Leu Gly Lys Gln Asp Arg Met Pro Thr  
 740                    745                    750

Ile Ile Leu Val Trp Ser Ile Leu Leu Ala Ser Ile Leu Thr Leu Met  
 755                    760                    765

Trp Val Arg Ile Asn Pro Phe Val Ser Arg Asp Gly Pro Val Leu Glu  
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Ile Cys Gly Leu Asn Cys Asp Glu Ser  
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 actgtctttt ttgtatattaa cttgaggggc ctggacggca ttcaaggacc agtttatgtg    180  
 ggaactgggtt gtgttttcaa cagaacggct atctatgggtt atgagcccc aattaaggcg    240  
 aagaagccag gtttcttggc atcattatgt nggggcaaga agaaggcaag caagtcaaag    300  
 aaaaggagct cagataagaa aaagtcaaac aagcatgtgg acagttctgt tccagtattc    360  
 aatctcaag acatagagga ggggtttgaa ggttctgggt ttgtatgtgaa gaaatcagtt    420  
 ctcatgtctc aaatgagctt agagaagaga tttggccagt cagcagcatt tggtgcctcc    480  
 actctgatgg aatatggtg tggatccatc tcgtccactc cagaatctct tttgaaagaa    540  
 gctatccatg tcataagttt tggctatgag gacaagtctg aatggggAAC tgagattgg    600  
 tggatctatg gatctgtcac agaagatatt ctaactggat tcaagatgca cgcaagaggc    660  
 tggcgttcaa tctattgtcat gcccaagcgc ccagcttca agggatctgc ccccatcaat    720  
 ctttcaagatc gctgtatca agtgcgtcggt tggctcttg gttctgttga aattcttttc    780  
 agccggcatt gccccttatg gtatggctac ggagggcgcc tcaagttctt ggagagattc    840  
 gcttacatca acaccaccaat ttacccacta acctctctcc cgcttcttagt ctattgtata    900  
 ttgcctgtca tctgtctgtc cactggaaag ttcatcatgc cagagattag caacttggcc    960  
 agtatctgtt tcattgtcgct cttccttca attttcgcca ctggatctct tgagatgagg    1020  
 tggagtgggtt ttggcattga cgagtgggtt aggaatgaac agttctgggtt cattggaggt    1080  
 atctctgtccc atctgtttgc cgtctttcag ggttctctgaa aggtgcttgc aggtatcgac    1140  
 accaacttca ctgtcaccc tc aaggctaat gatgaagaag ggcacttgc tgagctctac    1200  
 atgttcaagt ggacgacgct tcttacccct ccgacgacca ttttgcat taacatggtc    1260  
 ggtgtcgttg ctggtaatcc tc acggccatc aacagtgggtt accaactatg gggccgctc    1320

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 cttatggca gccaacccg cacaccgacg attgtcatcg tctggctgt cctccctcgct 1440  
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 aa 1742

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<212> PRT  
<213> Triticum aestivum

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Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg Asn Asp Arg  
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Tyr Ala Asn Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly Leu  
 35 40 45

Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe Asn  
 50 55 60

Arg Thr Ala Ile Tyr Gly Tyr Glu Pro Pro Ile Lys Ala Lys Lys Pro  
 65 70 75 80

Gly Phe Leu Ala Ser Leu Cys Xaa Gly Lys Lys Lys Ala Ser Lys Ser  
 85 90 95

Lys Lys Arg Ser Ser Asp Lys Lys Ser Asn Lys His Val Asp Ser  
 100 105 110

Ser Val Pro Val Phe Asn Leu Glu Asp Ile Glu Glu Gly Val Glu Gly  
 115 120 125

Ala Gly Phe Asp Asp Glu Lys Ser Val Leu Met Ser Gln Met Ser Leu  
 130 135 140

Glu Lys Arg Phe Gly Gln Ser Ala Ala Phe Val Ala Ser Thr Leu Met  
 145 150 155 160

Glu Tyr Gly Gly Val Pro Gln Ser Ser Thr Pro Glu Ser Leu Leu Lys  
 165 170 175

Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Ser Glu Trp  
 180 185 190

Gly Thr Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu  
 195 200 205

Thr Gly Phe Lys Met His Ala Arg Gly Trp Arg Ser Ile Tyr Cys Met  
 210 215 220

Pro Lys Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp  
 225                    230                    235                    240  
  
 Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Leu  
 245                    250                    255  
  
 Phe Ser Arg His Cys Pro Leu Trp Tyr Gly Tyr Gly Gly Arg Leu Lys  
 260                    265                    270  
  
 Phe Leu Glu Arg Phe Ala Tyr Ile Asn Thr Thr Ile Tyr Pro Leu Thr  
 275                    280                    285  
  
 Ser Leu Pro Leu Leu Val Tyr Cys Ile Leu Pro Ala Ile Cys Leu Leu  
 290                    295                    300  
  
 Thr Gly Lys Phe Ile Met Pro Glu Ile Ser Asn Leu Ala Ser Ile Trp  
 305                    310                    315                    320  
  
 Phe Ile Ala Leu Phe Leu Ser Ile Phe Ala Thr Gly Ile Leu Glu Met  
 325                    330                    335  
  
 Arg Trp Ser Gly Val Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe  
 340                    345                    350  
  
 Trp Val Ile Gly Gly Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly  
 355                    360                    365  
  
 Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser  
 370                    375                    380  
  
 Lys Ala Asn Asp Glu Glu Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys  
 385                    390                    395                    400  
  
 Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Met  
 405                    410                    415  
  
 Val Gly Val Val Ala Gly Thr Ser Tyr Ala Ile Asn Ser Gly Tyr Gln  
 420                    425                    430  
  
 Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile  
 435                    440                    445  
  
 Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg  
 450                    455                    460  
  
 Thr Pro Thr Ile Val Ile Val Trp Ala Val Leu Leu Ala Ser Ile Phe  
 465                    470                    475                    480  
  
 Ser Leu Leu Trp Val Arg Val Asp Pro Phe Thr Thr Arg Leu Ala Gly  
 485                    490                    495  
  
 Pro Asn Ile Gln Thr Cys Gly Ile Asn Cys  
 500                    505

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 <212> DNA  
 <213> Triticum aestivum

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tttcgcagat	cccaaccttc	cagtcaacc	gagatccatg	gaccgtcca	aggatctggc	180
ccgcctacgga	tatggcagcg	tggcctggaa	ggagagaatg	gagggcttga	agcagaagca	240
ggagcgcctg	cagcatgtca	ggagcgaggg	tggcggtgat	tggatggcg	acgatgcaga	300
tctgccacta	atggatgaag	ctaggcagcc	attgtccaga	aaagtcccta	tatcatcaag	360
ccgaattaat	ccctacagga	tgattatcgt	tatccggttg	gtggtttgg	gtttttctt	420
ccactaccca	gtgatgcata	cggcggaaaaga	tgcatttgca	ttgtggctca	tatctgtaat	480
ctgtgaaatc	tggtttgcga	tgtcctgtat	tctgtatcag	ttcccaaagt	gttttccaat	540
cgagagagag	acttacctgg	accgtttgtc	actaaggttt	gacaaggaaag	gtcaaccctc	600
tcagcttgct	ccaatcgact	tctttgtcag	tacgggtgat	cccacaaaagg	aacctccctt	660
ggtcacacgc	aacactgtcc	tttccatcct	ttctgtggat	tatccggttg	agaaggcttc	720
ctgctatgtt	tctgtatgatg	gtgctgcaat	gcttacgttt	gaagcattgt	ctgaaacatc	780
tgaatttgca	aagaaaatggg	ttccttcag	caaaaagttt	aatatcgagc	ctcgctgctcc	840
tgagtggtac	ttccaacaga	agatagacta	cctgaaaagac	aagggtgctg	cttcatttgt	900
tagggagagg	agggcgatga	agagagaata	cgaggaattc	aaggtaagga	tcaatgcctt	960
ggttgcaaaa	gccccaaaagg	ttcctgagga	aggatggaca	atgcaagatg	gaagcccttg	1020
gcctggaaaa						1029

&lt;210&gt; 22

&lt;211&gt; 340

&lt;212&gt; PRT

&lt;213&gt; Triticum aestivum

&lt;400&gt; 22

Pro	Leu	Leu	Thr	Asn	Gly	Gln	Met	Val	Asp	Asp	Ile	Pro	Pro	Glu	Gln
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His	Ala	Leu	Val	Pro	Ser	Tyr	Met	Ser	Gly	Gly	Gly	Gly	Gly	Lys
							20				25			30

Arg	Ile	His	Pro	Leu	Pro	Phe	Ala	Asp	Pro	Asn	Leu	Pro	Val	Gln	Pro
							35				40			45	

Arg	Ser	Met	Asp	Pro	Ser	Lys	Asp	Leu	Ala	Ala	Tyr	Gly	Tyr	Gly	Ser
						50				55				60	

Val	Ala	Trp	Lys	Glu	Arg	Met	Glu	Gly	Trp	Lys	Gln	Lys	Gln	Glu	Arg
						65				70				75	80

Leu	Gln	His	Val	Arg	Ser	Glu	Gly	Gly	Asp	Trp	Asp	Gly	Asp	Asp
						85				90			95	

Ala	Asp	Leu	Pro	Leu	Met	Asp	Glu	Ala	Arg	Gln	Pro	Leu	Ser	Arg	Lys
						100				105			110		

Val	Pro	Ile	Ser	Ser	Ser	Arg	Ile	Asn	Pro	Tyr	Arg	Met	Ile	Ile	Val
						115				120			125		

Ile	Arg	Leu	Val	Val	Leu	Gly	Phe	Phe	His	Tyr	Arg	Val	Met	His
						130				135			140	

Pro	Ala	Lys	Asp	Ala	Phe	Ala	Leu	Trp	Leu	Ile	Ser	Val	Ile	Cys	Glu
						145				150			155		160

Ile	Trp	Phe	Ala	Met	Ser	Cys	Ile	Leu	Asp	Gln	Phe	Pro	Lys	Trp	Phe
						165				170			175		

Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu Ser Leu Arg Phe Asp  
 180 185 190  
 Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile Asp Phe Phe Val Ser  
 195 200 205  
 Thr Val Asp Pro Thr Lys Glu Pro Pro Leu Val Thr Ala Asn Thr Val  
 210 215 220  
 Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Glu Lys Val Ser Cys Tyr  
 225 230 235 240  
 Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu Ala Leu Ser Glu  
 245 250 255  
 Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe Ser Lys Lys Phe Asn  
 260 265 270  
 Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln Gln Lys Ile Asp Tyr  
 275 280 285  
 Leu Lys Asp Lys Val Ala Ala Ser Phe Val Arg Glu Arg Arg Ala Met  
 290 295 300  
 Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val Ala  
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 Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Gln Asp Gly Ser  
 325 330 335  
 Pro Trp Pro Gly  
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 <213> Arabidopsis thaliana  
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 35 40 45  
 Ala Glu Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro  
 50 55 60  
 Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Cys  
 65 70 75 80  
 Cys Pro Gln Cys Lys Thr Arg Phe Arg Arg His Arg Gly Ser Pro Arg  
 85 90 95  
 Val Glu Gly Asp Glu Asp Glu Asp Asp Val Asp Asp Ile Glu Asn Glu  
 100 105 110

Phe Asn Tyr Ala Gln Gly Ala Asn Lys Ala Arg His Gln Arg His Gly  
 115 120 125  
 Glu Glu Phe Ser Ser Ser Arg His Glu Ser Gln Pro Ile Pro Leu  
 130 135 140  
 Leu Thr His Gly His Thr Val Ser Gly Glu Ile Arg Thr Pro Asp Thr  
 145 150 155 160  
 Gln Ser Val Arg Thr Thr Ser Gly Pro Leu Gly Pro Ser Asp Arg Asn  
 165 170 175  
 Ala Ile Ser Ser Pro Tyr Ile Asp Pro Arg Gln Pro Val Pro Val Arg  
 180 185 190  
 Ile Val Asp Pro Ser Lys Asp Leu Asn Ser Tyr Gly Leu Gly Asn Val  
 195 200 205  
 Asp Trp Lys Glu Arg Val Glu Gly Trp Lys Leu Lys Gln Glu Lys Asn  
 210 215 220  
 Met Leu Gln Met Thr Gly Lys Tyr His Glu Gly Lys Gly Glu Ile  
 225 230 235 240  
 Glu Gly Thr Gly Ser Asn Gly Glu Glu Leu Gln Met Ala Asp Asp Thr  
 245 250 255  
 Arg Leu Pro Met Ser Arg Val Val Pro Ile Pro Ser Ser Arg Leu Thr  
 260 265 270  
 Pro Tyr Arg Val Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe  
 275 280 285  
 Leu Gln Tyr Arg Thr Thr His Pro Val Lys Asn Ala Tyr Pro Leu Trp  
 290 295 300  
 Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Phe Ser Trp Leu Leu  
 305 310 315 320  
 Asp Gln Phe Pro Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp  
 325 330 335  
 Arg Leu Ala Ile Arg Tyr Asp Arg Asp Gly Glu Pro Ser Gln Leu Val  
 340 345 350  
 Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro  
 355 360 365  
 Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro  
 370 375 380  
 Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ser Ala Met Leu  
 385 390 395 400  
 Thr Phe Glu Ser Leu Ser Glu Thr Ala Glu Phe Ala Lys Lys Trp Val  
 405 410 415  
 Pro Phe Cys Lys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr  
 420 425 430

Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Ile Gin Pro Ser Phe  
 435 440 445  
 Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val  
 450 455 460  
 Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Ile Pro Glu Glu Gly  
 465 470 475 480  
 Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Thr Arg Asp  
 485 490 495  
 His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp  
 500 505 510  
 Thr Asp Gly Asn Glu Leu Pro Arg Leu Ile Tyr Val Ser Arg Glu Lys  
 515 520 525  
 Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu  
 530 535 540  
 Ile Arg Val Ser Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val  
 545 550 555 560  
 Asp Cys Asp His Tyr Phe Asn Asn Ser Lys Ala Ile Lys Glu Ala Met  
 565 570 575  
 Cys Phe Met Met Asp Pro Ala Ile Gly Lys Lys Cys Cys Tyr Val Gln  
 580 585 590  
 Phe Pro Gln Arg Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn  
 595 600 605  
 Arg Asn Ile Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile  
 610 615 620  
 Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala  
 625 630 635 640  
 Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Glu Asp Leu Glu Pro Asn  
 645 650 655  
 Ile Ile Val Lys Ser Cys Cys Gly Ser Arg Lys Lys Gly Lys Ser Ser  
 660 665 670  
 Lys Lys Tyr Asn Tyr Glu Lys Arg Arg Gly Ile Asn Arg Ser Asp Ser  
 675 680 685  
 Asn Ala Pro Leu Phe Asn Met Glu Asp Ile Asp Glu Gly Phe Glu Gly  
 690 695 700  
 Tyr Asp Asp Glu Arg Ser Ile Leu Met Ser Gln Arg Ser Val Glu Lys  
 705 710 715 720  
 Arg Phe Gly Gln Ser Pro Val Phe Ile Ala Ala Thr Phe Met Glu Gln  
 725 730 735  
 Gly Gly Ile Pro Pro Thr Thr Asn Pro Ala Thr Leu Leu Lys Glu Ala  
 740 745 750

Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys  
 755 760 765  
 Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly  
 770 775 780  
 Phe Lys Met His Ala Arg Gly Trp Ile Ser Ile Tyr Cys Asn Pro Pro  
 785 790 795 800  
 Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu  
 805 810 815  
 Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Leu Ser  
 820 825 830  
 Arg His Cys Pro Ile Trp Tyr Gly Tyr His Gly Arg Leu Arg Leu Leu  
 835 840 845  
 Glu Arg Ile Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile  
 850 855 860  
 Pro Leu Ile Ala Tyr Cys Ile Leu Pro Ala Phe Cys Leu Ile Thr Asp  
 865 870 875 880  
 Arg Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Ser Ile Trp Phe Ile  
 885 890 895  
 Leu Leu Phe Ile Ser Ile Ala Val Thr Gly Ile Leu Glu Leu Arg Trp  
 900 905 910  
 Ser Gly Val Ser Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val  
 915 920 925  
 Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu  
 930 935 940  
 Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala  
 945 950 955 960  
 Thr Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Ile Phe Lys Trp Thr  
 965 970 975  
 Ala Leu Leu Ile Pro Pro Thr Thr Val Leu Leu Val Asn Leu Ile Gly  
 980 985 990  
 Ile Val Ala Gly Val Ser Tyr Ala Val Asn Ser Gly Tyr Gln Ser Trp  
 995 1000 1005  
 Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Leu Trp Val Ile Ala His  
 1010 1015 1020  
 Leu Tyr Pro Phe Leu Lys Gly Leu Leu Gly Arg Gln Asn Arg Thr Pro  
 1025 1030 1035 1040  
 Thr Ile Val Ile Val Trp Ser Val Leu Leu Ala Ser Ile Phe Ser Leu  
 1045 1050 1055  
 Leu Trp Val Arg Ile Asn Pro Phe Val Asp Ala Asn Pro Asn Ala Asn  
 1060 1065 1070

Asn Phe Asn Gly Lys Gly Gly Val Phe  
       1075                  1080  
  
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       20                  25                  30  
  
 Glu Leu Ser Gly Gln Thr Cys Gln Ile Cys Gly Asp Glu Ile Glu Leu  
       35                  40                  45  
  
 Thr Val Ser Ser Glu Leu Phe Val Ala Cys Asn Glu Cys Ala Phe Pro  
       50                  55                  60  
  
 Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Asn Gln Ala  
       65                  70                  75                  80  
  
 Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Ile Lys Gly Ser Pro Arg  
       85                  90                  95  
  
 Val Asp Gly Asp Asp Glu Glu Glu Asp Ile Asp Asp Leu Glu Tyr  
       100                105                  110  
  
 Glu Phe Asp His Gly Met Asp Pro Glu His Ala Ala Glu Ala Ala Leu  
       115                120                  125  
  
 Ser Ser Arg Leu Asn Thr Gly Arg Gly Leu Asp Ser Ala Pro Pro  
       130                135                  140  
  
 Gly Ser Gln Ile Pro Leu Leu Thr Tyr Cys Asp Glu Asp Ala Asp Met  
       145                150                  155                  160  
  
 Tyr Ser Asp Arg His Ala Leu Ile Val Pro Pro Ser Thr Gly Tyr Gly  
       165                170                  175  
  
 Asn Arg Val Tyr Pro Ala Pro Phe Thr Asp Ser Ser Ala Pro Pro Gln  
       180                185                  190  
  
 Ala Arg Ser Met Val Pro Gln Lys Asp Ile Ala Glu Tyr Gly Tyr Gly  
       195                200                  205  
  
 Ser Val Ala Trp Lys Asp Arg Met Glu Val Trp Lys Arg Arg Gln Gly  
       210                215                  220  
  
 Glu Lys Leu Gln Val Ile Lys His Glu Gly Gly Asn Asn Gly Arg Gly  
       225                230                  235                  240  
  
 Ser Asn Asp Asp Asp Glu Leu Asp Asp Pro Asp Met Pro Met Met Asp  
       245                250                  255  
  
 Glu Gly Arg Gln Pro Leu Ser Arg Lys Leu Pro Ile Arg Ser Ser Arg  
       260                265                  270

Ile Asn Pro Tyr Arg Met Leu Ile Leu Cys Arg Leu Ala Ile Leu Gly  
 275 280 285  
 Leu Phe Phe His Tyr Arg Ile Leu His Pro Val Asn Asp Ala Tyr Gly  
 290 295 300  
 Leu Trp Leu Thr Ser Val Ile Cys Glu Ile Trp Phe Ala Val Ser Trp  
 305 310 315 320  
 Ile Leu Asp Gln Phe Pro Lys Trp Tyr Pro Ile Glu Arg Glu Thr Tyr  
 325 330 335  
 Leu Asp Arg Leu Ser Leu Arg Tyr Glu Lys Glu Gly Lys Pro Ser Gly  
 340 345 350  
 Leu Ala Pro Val Asp Val Phe Val Ser Thr Val Asp Pro Leu Lys Glu  
 355 360 365  
 Pro Pro Leu Ile Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp  
 370 375 380  
 Tyr Pro Val Asp Lys Val Ala Cys Tyr Val Ser Asp Asp Gly Ala Ala  
 385 390 395 400  
 Met Leu Thr Phe Glu Ala Leu Ser Asp Thr Ala Glu Phe Ala Arg Lys  
 405 410 415  
 Trp Val Pro Phe Cys Lys Phe Asn Ile Glu Pro Arg Ala Pro Glu  
 420 425 430  
 Trp Tyr Phe Ser Gln Lys Met Asp Tyr Leu Lys Asn Lys Val His Pro  
 435 440 445  
 Ala Phe Val Arg Glu Arg Arg Ala Met Lys Arg Asp Tyr Glu Glu Phe  
 450 455 460  
 Lys Val Lys Ile Asn Ala Leu Val Ala Thr Ala Gln Lys Val Pro Glu  
 465 470 475 480  
 Glu Gly Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val  
 485 490 495  
 Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly His Ser Gly Val  
 500 505 510  
 Arg Asp Thr Asp Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg  
 515 520 525  
 Glu Lys Arg Pro Gly Phe Asp His His Lys Lys Ala Gly Ala Met Asn  
 530 535 540  
 Ser Leu Ile Arg Val Ser Ala Val Leu Ser Asn Ala Pro Tyr Leu Leu  
 545 550 555 560  
 Asn Val Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Arg Glu  
 565 570 575  
 Ser Met Cys Phe Met Met Asp Pro Gln Ser Gly Lys Lys Val Cys Tyr  
 580 585 590

Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr  
 595 600 605  
 Ser Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp  
 610 615 620  
 Gly Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg  
 625 630 635 640  
 Gln Ala Leu Tyr Gly Phe Asp Ala Pro Lys Lys Lys Pro Pro Gly  
 645 650 655  
 Lys Thr Cys Asn Cys Trp Pro Lys Trp Cys Cys Leu Cys Cys Gly Leu  
 660 665 670  
 Arg Lys Lys Ser Lys Thr Lys Ala Lys Asp Lys Lys Thr Asn Thr Lys  
 675 680 685  
 Glu Thr Ser Lys Gln Ile His Ala Leu Glu Asn Val Asp Glu Gly Val  
 690 695 700  
 Ile Val Pro Val Ser Asn Val Glu Lys Arg Ser Glu Ala Thr Gln Leu  
 705 710 715 720  
 Lys Leu Glu Lys Lys Phe Gly Gln Ser Pro Val Phe Val Ala Ser Ala  
 725 730 735  
 Val Leu Gln Asn Gly Gly Val Pro Arg Asn Ala Ser Pro Ala Cys Leu  
 740 745 750  
 Leu Arg Glu Ala Ile Gln Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr  
 755 760 765  
 Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp  
 770 775 780  
 Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser Val Tyr  
 785 790 795 800  
 Cys Met Pro Lys Arg Ala Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu  
 805 810 815  
 Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu  
 820 825 830  
 Ile Phe Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly  
 835 840 845  
 Leu Lys Trp Leu Glu Arg Phe Ser Tyr Ile Asn Ser Val Val Tyr Pro  
 850 855 860  
 Trp Thr Ser Leu Pro Leu Ile Val Tyr Cys Ser Leu Pro Ala Val Cys  
 865 870 875 880  
 Leu Leu Thr Gly Lys Phe Ile Val Pro Glu Ile Ser Asn Tyr Ala Gly  
 885 890 895  
 Ile Leu Phe Met Leu Met Phe Ile Ser Ile Ala Val Thr Gly Ile Leu  
 900 905 910

Glu Met Gln Trp Gly Gly Val Gly Ile Asp Asp Trp Trp Arg Asn Glu  
 915 920 925  
 Gln Phe Trp Val Ile Gly Gly Ala Ser Ser His Leu Phe Ala Leu Phe  
 930 935 940  
 Gln Gly Leu Leu Lys Val Leu Ala Gly Val Asn Thr Asn Phe Thr Val  
 945 950 955 960  
 Thr Ser Lys Ala Ala Asp Asp Gly Ala Phe Ser Glu Leu Tyr Ile Phe  
 965 970 975  
 Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile Ile Asn  
 980 985 990  
 Ile Ile Gly Val Ile Val Gly Val Ser Asp Ala Ile Ser Asn Gly Tyr  
 995 1000 1005  
 Asp Ser Trp Gly Pro Leu Phe Gly Arg Leu Phe Phe Ala Leu Trp Val  
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 Ile Val His Leu Tyr Pro Phe Leu Lys Gly Met Leu Gly Lys Gln Asp  
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 Lys Met Pro Thr Ile Ile Val Val Trp Ser Ile Leu Leu Ala Ser Ile  
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 His Pro Ser Phe Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu  
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 Pro Glu Glu Gly Trp Val Met Gln Asp Gly Thr Pro Trp Pro Gly Asn  
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 Asn Thr Arg Asp His Pro Gly Met Ile Gln Val Tyr Leu Gly Ser Ala  
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 Gly Ala Leu Asp Val Asp Gly Lys Glu Leu Pro Arg Leu Val Tyr Val  
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Ser Arg Glu Lys Arg Pro Gly Tyr Gln His His Lys Lys Ala Gly Ala  
 115 120 125  
 Glu Asn Ala Leu Val Arg Val Ser Ala Val Leu Thr Asn Ala Pro Phe  
 130 135 140  
 Ile Leu Asn Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Met  
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 Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp  
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 Arg Tyr Ala Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Leu Gly  
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 210 215 220  
 Asn Arg Gln Ala Leu Tyr Gly Tyr Asp Pro Pro Val Ser Glu Lys Arg  
 225 230 235 240  
 Pro Lys Met Thr Cys Asp Cys Trp Pro Ser Trp Cys Cys Cys Cys Cys  
 245 250 255  
 Gly Gly Ser Arg Lys Lys Ser Lys Lys Lys Gly Glu Lys Lys Gly Leu  
 260 265 270  
 Leu Gly Gly Leu Leu Tyr Gly Lys Lys Lys Lys Met Met Gly Lys Asn  
 275 280 285  
 Tyr Val Lys Lys Gly Ser Ala Pro Val Phe Asp Leu Glu Glu Ile Glu  
 290 295 300  
 Glu Gly Leu Glu Gly Tyr Glu Glu Leu Glu Lys Ser Thr Leu Met Ser  
 305 310 315 320  
 Gln Lys Asn Phe Glu Lys Arg Phe Gly Gln Ser Pro Val Phe Ile Ala  
 325 330 335  
 Ser Thr Leu Met Glu Asn Gly Gly Leu Pro Glu Gly Thr Asn Ser Thr  
 340 345 350  
 Ser Leu Ile Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Glu  
 355 360 365  
 Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr  
 370 375 380  
 Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys Arg Gly Trp Lys Ser  
 385 390 395 400  
 Val Tyr Cys Val Pro Lys Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile  
 405 410 415  
 Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser  
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Val Glu Ile Phe Leu Ser Arg His Cys Pro Leu Trp Tyr Gly Tyr Gly  
                   435                  440                  445  
 Gly Lys Leu Lys Trp Leu Glu Arg Leu Ala Tyr Ile Asn Thr Ile Val  
                   450                  455                  460  
 Tyr Pro Phe Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Ile Pro Ala  
                   465                  470                  475                  480  
 Val Cys Leu Leu Thr Gly Lys Phe Ile Ile Pro Thr Leu Ser Asn Leu  
                   485                  490                  495  
 Thr Ser Val Trp Phe Leu Ala Leu Phe Leu Ser Ile Ile Ala Thr Gly  
                   500                  505                  510  
 Val Leu Glu Leu Arg Trp Ser Gly Val Ser Ile Gln Asp Trp Trp Arg  
                   515                  520                  525  
 Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ala His Leu Phe Ala  
                   530                  535                  540  
 Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Val Asp Thr Asn Phe  
                   545                  550                  555                  560  
 Thr Val Thr Ala Lys Ala Ala Asp Asp Thr Glu Phe Gly Glu Leu Tyr  
                   565                  570                  575  
 Leu Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Ile Ile  
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 Leu Asn Met Val Gly Val Val Ala Gly Val Ser Asp Ala Ile Asn Asn  
                   595                  600                  605  
 Gly Tyr Gly Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe  
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 Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg  
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 Gln Asn Arg Thr Pro Thr Ile Val Val Leu Trp Ser Ile Leu Leu Ala  
                   645                  650                  655  
 Ser Ile Phe Ser Leu Val Trp Val Arg Ile Asp Pro Phe Leu Pro Lys  
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Tyr Val Ser Leu Ser Arg Asp Asn Ile Glu Leu Ser Gly Glu Leu Ser  
                   35                  40                  45  
 Gly Asp Tyr Ser Asn Tyr Thr Val His Ile Pro Pro Thr Pro Asp Asn  
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 Gln Pro Met Ala Thr Lys Ala Glu Glu Gln Tyr Val Ser Asn Ser Leu  
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 Phe Thr Gly Gly Phe Asn Ser Val Thr Arg Ala His Leu Met Asp Lys  
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 Val Ile Asp Ser Asp Val Thr His Pro Gln Met Ala Gly Ala Lys Gly  
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 Ser Ser Cys Ala Met Pro Ala Cys Asp Gly Asn Val Met Lys Asp Glu  
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                   130                135                  140  
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 Lys Glu Gln Tyr Lys Ile Gly Asp Leu Asp Asp Asp Thr Pro Asp Tyr  
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 Ser Ser Gly Ala Leu Pro Leu Pro Ala Pro Gly Lys Asp Gln Arg Gly  
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 Asp His Asn Arg Trp Leu Phe Glu Thr Gln Gly Thr Tyr Gly Tyr Gly  
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                   225                230                  235                  240  
 Gly Met Arg Gly Gly Met Val Glu Thr Ala Asp Lys Pro Trp Arg Pro  
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 Leu Ser Arg Arg Ile Pro Ile Pro Ala Ala Ile Ile Ser Pro Tyr Arg  
                   260                265                  270  
 Leu Leu Ile Val Ile Arg Phe Val Val Leu Cys Phe Phe Leu Thr Trp  
                   275                280                  285  
 Arg Ile Arg Asn Pro Asn Glu Asp Ala Ile Trp Leu Trp Leu Met Ser  
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 Ile Ile Cys Glu Leu Trp Phe Gly Phe Ser Trp Ile Leu Asp Gln Ile  
                   305                310                  315                  320  
 Pro Lys Leu Cys Pro Ile Asn Arg Ser Thr Asp Leu Glu Val Leu Arg  
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 Asp Lys Phe Asp Met Pro Ser Pro Ser Asn Pro Thr Gly Arg Ser Asp  
                   340                345                  350

Leu Pro Gly Ile Asp Leu Phe Val Ser Thr Ala Asp Pro Glu Lys Glu  
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 Tyr Pro Val Glu Lys Val Ser Cys Tyr Leu Ser Asp Asp Gly Gly Ala  
 385 390 395 400  
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 Trp Val Pro Phe Cys Arg Lys His Asn Ile Glu Pro Arg Asn Pro Asp  
 420 425 430  
 Ser Tyr Phe Ser Leu Lys Ile Asp Pro Thr Lys Asn Lys Ser Arg Ile  
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 Lys Val Arg Ile Asn Gly Leu Pro Asp Ser Ile Arg Arg Arg Ser Asp  
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 Ala Phe Asn Ala Arg Glu Glu Met Lys Ala Leu Lys Gln Met Arg Glu  
 485 490 495  
 Ser Gly Gly Asp Pro Thr Glu Pro Val Lys Val Pro Lys Ala Thr Trp  
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 Glu His Ser Lys Gly Asp His Ala Gly Ile Leu Gln Val Met Leu Lys  
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 Pro Pro Ser Ser Asp Pro Leu Ile Gly Asn Ser Asp Asp Lys Val Ile  
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 Asp Phe Ser Asp Thr Asp Thr Arg Leu Pro Met Phe Val Tyr Val Ser  
 565 570 575  
 Arg Glu Lys Arg Pro Gly Tyr Asp His Asn Lys Lys Ala Gly Ala Met  
 580 585 590  
 Asn Ala Leu Val Arg Ala Ser Ala Ile Leu Ser Asn Gly Pro Phe Ile  
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 Leu Asn Leu Asp Cys Asp His Tyr Ile Tyr Asn Cys Lys Ala Val Arg  
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 Glu Gly Met Cys Phe Met Met Asp Arg Gly Gly Glu Asp Ile Cys Tyr  
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 Ile Gln Phe Pro Gln Arg Phe Glu Gly Ile Asp Pro Ser Asp Arg Tyr  
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 Ala Asn Asn Asn Thr Val Phe Phe Asp Gly Asn Met Arg Ala Leu Asp  
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Gly Val Gln Gly Pro Val Tyr Val Gly Thr Gly Thr Met Phe Arg Arg  
 675 680 685  
 Phe Ala Leu Tyr Gly Phe Asp Pro Pro Asn Pro Asp Lys Leu Leu Glu  
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 705 710 715 720  
 Asp Leu Asp Val Thr Gln Leu Pro Lys Arg Phe Gly Asn Ser Thr Leu  
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 Leu Ala Glu Ser Ile Pro Ile Ala Glu Phe Gln Gly Arg Pro Leu Ala  
 740 745 750  
 Asp His Pro Ala Val Lys Tyr Gly Arg Pro Pro Gly Ala Leu Arg Val  
 755 760 765  
 Pro Arg Asp Pro Leu Asp Ala Thr Thr Val Ala Glu Ser Val Ser Val  
 770 775 780  
 Ile Ser Cys Trp Tyr Glu Asp Lys Thr Glu Trp Gly Asp Arg Val Gly  
 785 790 795 800  
 Trp Ile Tyr Gly Ser Val Thr Glu Asp Val Val Thr Gly Tyr Arg Met  
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 His Asn Arg Gly Trp Arg Ser Val Tyr Cys Ile Thr Lys Arg Asp Ser  
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 Phe Arg Gly Ser Ala Pro Ile Asn Leu Thr Asp Arg Leu His Gln Val  
 835 840 845  
 Leu Arg Trp Ala Thr Gly Ser Val Glu Ile Phe Phe Ser Arg Asn Asn  
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 885 890 895  
 Cys Phe Leu Pro Ala Phe Ser Leu Phe Ser Gly Gln Phe Ile Val Arg  
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 Thr Leu Ser Ile Ser Phe Leu Val Tyr Leu Leu Met Ile Thr Ile Cys  
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 Leu Ile Gly Leu Ala Val Leu Glu Val Lys Trp Ser Gly Ile Gly Leu  
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 Glu Glu Trp Trp Arg Asn Glu Gln Trp Trp Leu Ile Ser Gly Thr Ser  
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 Ser His Leu Tyr Ala Val Val Gln Gly Val Leu Lys Val Ile Ala Gly  
 965 970 975  
 Ile Glu Ile Ser Phe Thr Leu Thr Thr Lys Ser Gly Gly Asp Asp Asn  
 980 985 990

Glu Asp Ile Tyr Ala Asp Leu Tyr Ile Val Lys Trp Ser Ser Leu Met  
 995 1000 1005  
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 Phe Val Trp Ala Gly Leu Ile Ala Ile Thr Ile Ser Leu Leu Trp Thr  
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 35 40 45  
 Glu Gly Asp Leu Phe Val Ala Cys Asn Glu Cys Gly Phe Pro Ala Cys  
 50 55 60  
 Arg Pro Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Thr Gln Asn Cys Pro  
 65 70 75 80  
 Gln Cys Lys Thr Arg Tyr Lys Arg Leu Arg Gly Ser Pro Arg Val Glu  
 85 90 95  
 Gly Asp Glu Asp Glu Glu Asp Ile Asp Asp Ile Glu Tyr Glu Phe Asn  
 100 105 110  
 Ile Glu His Glu Gln Asp Lys His Lys His Ser Ala Glu Ala Met Leu  
 115 120 125  
 Tyr Gly Lys Met Ser Tyr Gly Arg Gly Pro Glu Asp Asp Glu Asn Gly  
 130 135 140  
 Arg Phe Pro Pro Val Ile Ala Gly Gly His Ser Gly Glu Phe Pro Val  
 145 150 155 160

Gly Gly Gly Tyr Gly Asn Gly Glu His Gly Leu His Lys Arg Val His  
 165 170 175

Pro Tyr Pro Ser Ser Glu Ala Gly Ser Glu Gly Gly Trp Arg Glu Arg  
 180 185 190

Met Asp Asp Trp Lys Leu Gln His Gly Asn Leu Gly Pro Glu Pro Asp  
 195 200 205

Asp Asp Pro Glu Met Gly Leu Ile Asp Glu Ala Arg Gln Pro Leu Ser  
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Arg Lys Val Pro Ile Ala Ser Ser Lys Ile Asn Pro Tyr Arg Met Val  
 225 230 235 240

Ile Val Ala Arg Leu Val Ile Leu Ala Val Phe Leu Arg Tyr Arg Leu  
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Leu Asn Pro Val His Asp Ala Leu Gly Leu Trp Leu Thr Ser Val Ile  
 260 265 270

Cys Glu Ile Trp Phe Ala Val Ser Trp Ile Leu Asp Gln Phe Pro Lys  
 275 280 285

Trp Phe Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu Ser Leu Arg  
 290 295 300

Tyr Glu Arg Glu Gly Glu Pro Asn Met Leu Ala Pro Val Asp Val Phe  
 305 310 315 320

Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val Thr Ser Asn  
 325 330 335

Thr Val Leu Ser Ile Leu Ala Met Asp Tyr Pro Val Glu Lys Ile Ser  
 340 345 350

Cys Tyr Val Ser Asp Asp Gly Ala Ser Met Leu Thr Phe Glu Ser Leu  
 355 360 365

Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys Lys  
 370 375 380

Phe Ser Ile Glu Pro Arg Ala Pro Glu Met Tyr Phe Thr Leu Lys Val  
 385 390 395 400

Asp Tyr Leu Gln Asp Lys Val His Pro Thr Phe Val Lys Glu Arg Arg  
 405 410 415

Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Gln  
 420 425 430

Val Ala Lys Ala Ser Lys Val Pro Leu Glu Gly Trp Ile Met Gln Asp  
 435 440 445

Gly Thr Pro Trp Pro Gly Asn Asn Thr Lys Asp His Pro Gly Met Ile  
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Gln Val Phe Leu Gly His Ser Gly Gly Phe Asp Val Glu Gly His Glu  
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Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe Gln  
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 Pro Gln Ile Gly Lys Lys Val Cys Tyr Val Gln Phe Pro Gln Arg Phe  
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                   565                  570                  575  
  
 Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr  
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 Val Gly Thr Gly Cys Val Phe Lys Arg Gln Ala Leu Tyr Gly Tyr Glu  
                   595                  600                  605  
  
 Pro Pro Lys Gly Pro Lys Arg Pro Lys Met Ile Ser Cys Gly Cys Cys  
                   610                  615                  620  
  
 Pro Cys Phe Gly Arg Arg Lys Asn Lys Lys Phe Ser Lys Asn Asp  
                   625                  630                  635                  640  
  
 Met Asn Gly Asp Val Ala Ala Leu Gly Ala Glu Gly Asp Lys Glu  
                   645                  650                  655  
  
 His Leu Met Phe Glu Met Asn Phe Glu Lys Thr Phe Gly Gln Ser Ser  
                   660                  665                  670  
  
 Ile Phe Val Thr Ser Thr Leu Met Glu Glu Gly Val Pro Pro Ser  
                   675                  680                  685  
  
 Ser Ser Pro Ala Val Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys  
                   690                  695                  700  
  
 Gly Tyr Glu Asp Lys Thr Glu Trp Gly Thr Glu Leu Gly Trp Ile Tyr  
                   705                  710                  715                  720  
  
 Gly Ser Ile Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys Arg  
                   725                  730                  735  
  
 Gly Trp Arg Ser Ile Tyr Cys Met Pro Lys Arg Pro Ala Phe Lys Gly  
                   740                  745                  750  
  
 Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp  
                   755                  760                  765  
  
 Ala Leu Gly Ser Val Glu Ile Phe Phe Ser Arg His Ser Pro Leu Trp  
                   770                  775                  780  
  
 Tyr Gly Tyr Lys Gly Lys Leu Lys Trp Leu Glu Arg Phe Ala Tyr  
                   785                  790                  795                  800

Ala Asn Thr Thr Ile Tyr Pro Phe Thr Ser Ile Pro Leu Leu Ala Tyr  
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 Cys Ile Leu Pro Ala Ile Cys Leu Leu Thr Asp Lys Phe Ile Met Pro  
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 Pro Ile Ser Thr Phe Ala Ser Leu Phe Phe Ile Ser Leu Phe Met Ser  
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 Ile Ile Val Thr Gly Ile Leu Glu Leu Arg Trp Ser Gly Val Ser Ile  
 850 855 860  
 Glu Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Ile Ser  
 865 870 875 880  
 Ala His Leu Phe Ala Val Val Gln Gly Leu Leu Lys Ile Leu Ala Gly  
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 Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Thr Asp Asp Asp Asp  
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 Phe Gly Glu Leu Tyr Ala Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro  
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 Thr Thr Val Leu Ile Ile Asn Ile Val Gly Val Val Ala Gly Ile Ser  
 930 935 940  
 Asp Ala Ile Asn Asn Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys  
 945 950 955 960  
 Leu Phe Phe Ser Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys  
 965 970 975  
 Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Val Ile Trp  
 980 985 990  
 Ser Val Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp  
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Glu Trp Tyr Phe Ala Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Gln  
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Thr Ser Phe Val Lys Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu  
       65                  70                  75                  80

Phe Lys Val Arg Val Asn Gly Leu Val Ala Lys Ala Gln Lys Val Pro  
       85                  90                  95

Glu Glu Gly Trp Ile Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn  
       100                105                  110

Thr Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly  
       115                120                  125

Gly Leu Asp Ala Glu Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser  
       130                135                  140

Arg Glu Lys Arg Pro Gly Phe Gln His His Lys Lys Ala Gly Ala Met  
       145                150                  155                  160

Asn Ala Leu Val Arg Val Ser Ala Val Leu Thr Asn Gly Ala Phe Leu  
       165                170                  175

Leu Asn Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Leu Arg  
       180                185                  190

Glu Ala Met Cys Phe Leu Met Asp Pro Asn Leu Gly Lys Gln Val Cys  
       195                200                  205

Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg Asn Asp Arg  
       210                215                  220

Tyr Ala Asn Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly Leu  
       225                230                  235                  240

Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe Asn  
       245                250                  255

Arg Thr Ala Leu Tyr Gly Tyr Glu Pro Pro Leu Lys Pro Lys His Arg  
       260                265                  270

Lys Thr Gly Ile Leu Ser Ser Leu Cys Gly Gly Ser Arg Lys Lys Ser  
       275                280                  285

Ser Lys Ser Ser Lys Lys Gly Ser Asp Lys Lys Lys Ser Gly Lys His  
       290                295                  300

Val Asp Ser Thr Val Pro Val Phe Asn Leu Glu Asp Ile Glu Glu Gly  
       305                310                  315                  320

Val Glu Gly Ala Gly Phe Asp Asp Glu Lys Ser Leu Leu Met Ser Gln  
       325                330                  335

Met Ser Leu Glu Lys Arg Phe Gly Gln Ser Ala Val Phe Val Ala Ser  
       340                345                  350

Thr Leu Met Glu Asn Gly Gly Val Pro Gln Ser Ala Thr Pro Glu Thr  
       355                360                  365

Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys  
 370 375 380  
 Thr Asp Trp Gly Ser Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu  
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 Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg Gly Trp Arg Ser Ile  
 405 410 415  
 Tyr Cys Met Pro Lys Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn  
 420 425 430  
 Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Val  
 435 440 445  
 Glu Ile Leu Phe Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Ser Gly  
 450 455 460  
 Arg Leu Lys Trp Leu Glu Arg Phe Ala Tyr Val Asn Thr Thr Ile Tyr  
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 Pro Val Thr Ala Ile Pro Leu Leu Met Tyr Cys Thr Leu Pro Ala Val  
 485 490 495  
 Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Gln Ile Ser Asn Leu Ala  
 500 505 510  
 Ser Ile Trp Phe Ile Ser Leu Phe Leu Ser Ile Phe Ala Thr Gly Ile  
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 Leu Lys Met Lys Trp Asn Gly Val Gly Ile Asp Gln Trp Trp Arg Asn  
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 Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ala His Leu Phe Ala Val  
 545 550 555 560  
 Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr  
 565 570 575  
 Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr  
 580 585 590  
 Met Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Ile  
 595 600 605  
 Ile Asn Leu Val Gly Val Val Ala Gly Ile Ser Tyr Val Ile Asn Ser  
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 Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe  
 625 630 635 640  
 Trp Val Ile Ile His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg  
 645 650 655  
 Gln Asn Arg Thr Pro Thr Ile Val Val Val Trp Ser Ile Leu Leu Ala  
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Thr Ser Leu Leu Ile Pro Pro Met Thr Leu Leu Ile Ile Asn Val Ile  
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995 1000 1005

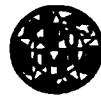
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1025 1030 1035 1040

Pro Thr Ile Ile Val Val Trp Ser Ile Leu Leu Ala Ser Ile Leu Thr  
1045 1050 1055

Leu Leu Trp Val Arg Val Asn Pro Phe Val Ala Lys Gly Gly Pro Ile  
1060 1065 1070

Leu Glu Ile Cys Gly Leu Asp Cys Leu  
1075 1080



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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			(43) International Publication Date: <b>27 January 2000 (27.01.00)</b>
<p>(21) International Application Number: <b>PCT/US99/15871</b></p> <p>(22) International Filing Date: <b>13 July 1999 (13.07.99)</b></p> <p>(30) Priority Data: <b>60/092,844</b>      <b>14 July 1998 (14.07.98)</b>      <b>US</b></p> <p>(71) Applicant (<i>for all designated States except US</i>): <b>E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).</b></p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): <b>ALLEN, Stephen, M. [US/US]; 2225 Rosewood Drive, Wilmington, DE 19810 (US). FADER, Gary, M. [US/US]; 1000 Woods Lane, Landenberg, PA 19350 (US). FALCO, Saverio, Carl [US/US]; 1902 Millers Road, Arden, DE 19810 (US). KINNEY, Anthony, J. [GB/US]; 609 Lore Avenue, Wilmington, DE 19809 (US). LIGHTNER, Jonathan, E. [US/US]; 4180 Delta Road, Airville, PA 17302 (US). MIAO, Guo-Hua [CN/US]; 202 Cherry Blossom Place, Hockessin, DE 19707 (US). RAFALSKI, J., Antoni [US/US]; 2028 Longcome Drive, Wilmington, DE 19810 (US). THORPE, Catherine, J. [GB/GB]; 120 Ross Street, Cambridge CB1 3BU (GB).</b></p>		<p>(74) Agent: <b>MAJARIAN, William, R.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).</b></p> <p>(81) Designated States: <b>AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b></p> <p><b>Published</b> <i>With international search report.</i></p> <p>(88) Date of publication of the international search report: <b>27 April 2000 (27.04.00)</b></p>	
<p>(54) Title: <b>PLANT CELLULOSE SYNTHASES</b></p> <p>(57) Abstract</p> <p>This invention relates to an isolated nucleic acid fragment encoding a cellulose synthase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the cellulose synthase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the cellulose synthase in a transformed host cell.</p> <p>SEQ ID NO:2 1 60    SEQ ID NO:4 RAAGAGRHHKPPKPKQKLAVSLP—LPHEHFTFVVFVAKTRK—KTAACPG—    SEQ ID NO:6 KSTTYTKERELAQPAAAPKQPP—ATACACRSRPPDQQRGGGLRAFNCANAQDV    SEQ ID NO:8    SEQ ID NO:10    SEQ ID NO:12 RCB—KMTCCSPTTTTPTKLLKPLPT    SEQ ID NO:14    SEQ ID NO:16    SEQ ID NO:18    SEQ ID NO:20    SEQ ID NO:22    SEQ ID NO:23    SEQ ID NO:24 HSTYGR—    SEQ ID NO:25    SEQ ID NO:26 HASTPPOTSEKVNSHNGGSGQTVKFAKRTSSQTVSLP—KONIELSGELSDGTNTTVKIP    SEQ ID NO:27    SEQ ID NO:28    SEQ ID NO:29 R—PR—    61 120    SEQ ID NO:2 N—REGARD—ME—ASAGLVAVSSINRHLV—VIRBRGEPGPKP—MDRNGIVCQI—    SEQ ID NO:4    SEQ ID NO:6    SEQ ID NO:8 KERDPAGRGGSPTE—ASAGLVAVSSINRHLV—VIRBRGEPGPKP—MDRNGIVCQI—    SEQ ID NO:10 ME—ASAGLVAVSSINRHLV—VIRBRGEPGPKP—MDRNGIVCQI—    SEQ ID NO:12    SEQ ID NO:14 ME—ASAGLVAVSSINRHLV—VIRBRGEPGPKP—MDRNGIVCQI—    SEQ ID NO:16    SEQ ID NO:18    SEQ ID NO:20    SEQ ID NO:22    SEQ ID NO:23    SEQ ID NO:24    SEQ ID NO:25    SEQ ID NO:26 PTPONOMATAKETOTVSHMLFTGSGTNTVTRAHLMEDVVIDEUVTHPOMAGAMHRCAMP    SEQ ID NO:27    SEQ ID NO:28    SEQ ID NO:29 ME—ASAGLVAVSSINRHLV—VIRBRGEPGPKP—MDRNGIVCQI—    121 180    SEQ ID NO:2 CDDOWHNPDSSTPVVACHECAFPZICHCYTYEERGDTOPCPQCNTTFLMLMCANP60—    SEQ ID NO:4    SEQ ID NO:6    SEQ ID NO:8    SEQ ID NO:10 CDRHVVYGDSEPVVACHECAFPVVCACYTYEERGDTOPCPQCNTTFLMLMCANP60—    SEQ ID NO:12    SEQ ID NO:14 CDRHVVYGDSEPVVACHECAFPVVCACYTYEERGDTOPCPQCNTTFLMLMCANP60—    SEQ ID NO:16    SEQ ID NO:18    SEQ ID NO:20    SEQ ID NO:22    SEQ ID NO:23 CDRHVVYGDSEPVVACHECAFPVVCACYTYEERGDTOPCPQCNTTFLMLMCANP60—    SEQ ID NO:24    SEQ ID NO:25 CDRHVVYGDSEPVVACHECAFPVVCACYTYEERGDTOPCPQCNTTFLMLMCANP60—    SEQ ID NO:26 CDRHVVYGDSEPVVACHECAFPVVCACYTYEERGDTOPCPQCNTTFLMLMCANP60—</p>			

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## INTERNATIONAL SEARCH REPORT

International Application No PCT, US 99/15871
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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54	C12N1/21	C12N9/10	C12Q1/48	C12Q1/68
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According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 00549 A (WILLIAMSON RICHARD EDWARD ; PENG LIANGCAI (AU); ARIOLI ANTONIO (AU)) 8 January 1998 (1998-01-08) see SEQ ID NOS:1-12</p> <p>---</p> <p>-/-</p>	1,2,4-7, 10-17



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

9 February 2000

Date of mailing of the international search report

23. 02. 00

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Maddox, A

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT, US 99/15871

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARIOLI, T., ET AL.: "Arabidopsis thaliana cellulose synthase catalytic subunit (RSW1) gene complete cds" EMBL ACCESSION NO:AF027172, 3 February 1998 (1998-02-03), XP002124282 the whole document	1,4-6, 13-17
X	-& ARIOLI, T. ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, 30 January 1998 (1998-01-30), pages 717-720, XP002124283 the whole document & ARIOLI, T., ET AL.: "Cellulose synthase catalytic subunit" TREMBL ACCESSION NO:048946, 1 June 1998 (1998-06-01), ---	6,13-17
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	& PEAR, J.R., ET AL.: "Cellulose synthase fragment" TREMBL ACCESSION NO:P93156, 1 May 1997 (1997-05-01), ---	1,2,4-6
X	PEAR, J.R., ET AL.: "Gossypium hirsutum cellulose synthase (celA1) mRNA, complete cds" EMBL ACCESSION NO:U58283, 13 December 1996 (1996-12-13), XP002124439 the whole document	1,2,4-6, 13-17
X	-& PEAR, J.R., ET AL.: "HIGHER PLANTS CONTAIN HOMOLOGS OF THE BACTERIAL CELA GENES ENCODING THE CATALYTIC SUBUNIT OF CELLULOSE SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, October 1996 (1996-10), pages 12637-12642, XP002061424 the whole document & PEAR, J.R. ET AL.: "Cellulose synthase" TREMBL ACCESSION NO:P93155, 1 May 1997 (1997-05-01), ---	6,13-17
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X	<p>DBEST DATABASE ID:37681,            2 December 1993 (1993-12-02), XP002124440            the whole document</p> <p>&amp; SASAKI, T.: "Rice cDNA, partial sequence (R1814-1A)"            EMBL ACCESSION NO:D24381,            29 November 1993 (1993-11-29),</p> <p>---</p>	1,2,4-6, 16,17
X	<p>DATABASE DBEST ID:1473188,            20 January 1998 (1998-01-20), XP002129996            &amp; NAHM, B.H., ET AL.: "96AS0237 Rice Immature Seed Lambda ZAPII cDNA Library Oryza sativa cDNA clone 96AS0237."            EMBL ACCESSION NO:AA751514,            21 January 1998 (1998-01-21),</p> <p>---</p>	1,2,4-6, 16,17
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International Application No

PL., US 99/15871

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 00551 A (MAYO FOUNDATION ;MCDONALD JOHN A (US); SPICER ANDREW P (US); AUGUS) 8 January 1998 (1998-01-08) see page 14 lines 21 and 22, and SEQ ID NO:48 ----	6
P,X	EP 0 875 575 A (NISSHIN SPINNING) 4 November 1998 (1998-11-04) the whole document ----	1,2,4-7, 10-12
P,X	SASAKI, T., ET AL.: "Oryza sativa cDNA, partial sequence (R2825_6A)." EMBL ACCESSION NO:AU031954, 19 October 1998 (1998-10-19), XP002129999 the whole document ----	16,17
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 723 764 A (SINGLETARY GEORGE WILLIAM ET AL) 3 March 1998 (1998-03-03) ---	13
A	AMOR Y ET AL: "EVIDENCE FOR A CYCLIC DIGUANYLIC ACID-DEPENDENT CELLULOSE SYNTHASE IN PLANTS" PLANT CELL, US, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 3, page 989-995 XP002061420 ISSN: 1040-4651 the whole document ---	6,18
A	LI ET AL: "beta-Glucan synthesis in the cotton fiber" PLANT PHYSIOLOGY, US, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 101, no. 4, page 1149-1156 XP002087180 ISSN: 0832-0889 the whole document ---	6,18
A	WO 91 13988 A (UNIV TEXAS) 19 September 1991 (1991-09-19) the whole document -----	1-6, 13-18

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/15871

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheets

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding barley cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOS:1 and 2, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

2. Claims: 1-6,13-18 all partially and 7-12 all completely

Nucleic acid fragments encoding corn cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOS:3-10, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

3. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding rice cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOS:11 and 12, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

4. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding soybean cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOS:13-18, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

5. Claims: 1-6,13-18 all partially

Nucleic acid fragments encoding wheat cellulose synthase, and corresponding polypeptide as represented by SEQ ID NOS:19-22, fragments encoding amino acid sequence 90% identical thereto, transformed hosts containing and methods for obtaining said sequences, methods for altering expression and methods for evaluating inhibitors using said sequences.

6. Claim : 18 partially

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Method for evaluating a compound for inhibitory activity on cellulose synthase comparing activity of cellulose synthase produced in a transformed host with and without the addition of the compound, not covered by any of the previous groups of claimed inventions 1-5.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PL, US 99/15871

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
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